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GENETIC STABILITY OF HAPLOID, DIPLOID, AND TETRAPLOID GENOTYPES IN THE TOMATO[1]

E. W. LINDSTROM
Iowa State College, Ames, Iowa

Received December 14, 1940

THE spontaneous origin in 1926 of a tomato haploid in *Lycopersicon esculentum* Mill. (LINDSTROM 1929) provided the source material of these investigations. This haploid when doubled asexually by the decapitation-callus method becomes an absolutely homozygous diploid; and doubling this in turn results in the homozygous tetraploid. Selection experiments have been in progress since 1926 in the haploid, since 1929 in the diploid, and since 1930 in the tetraploid. Cytological descriptions of these tomato forms appear in earlier reports (HUMPHREY 1934, 1937; LINDSTROM and KOOS 1931; LINDSTROM and HUMPHREY 1933).

The main objective of the selection experiments was to test the relative stability of the tomato genotype in the n, 2n, and 4n condition of comparable material. An original haploid plant may be continued indefinitely from cuttings. In the 2n and 4n condition of absolutely homozygous genotypes, there is an exceptionally good genetic basis for selection problems.

EXPERIMENTAL

Haploid

Since 1926 this 12-chromosome form has been maintained by cuttings, usually three to four crops per year, each crop of cuttings consisting of 30 to 100 plants. These are grown to maturity in eight inch greenhouse pots. Thus the haploid has been under observation for 14 years, involving a minimum of 2000 individual mature plants from cuttings, not including many hundreds of cuttings carried as reserves or as experimental plants for other investigations.

Fertility

During this time fertility has not changed. The haploid is still highly sterile, setting only an occasional fruit naturally. Artificial selfing at various periods produced a total of only 14 viable seeds which proved to be normal diploids; and outcrosses to diploid male plants yielded a number of seeds not inconsistent with a random inclusion of all 12 chromosomes in an occasional egg.

It was expected that full fertility might automatically arise if natural doubling of chromosomes occurred in meristematic micro- and megaspore

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producing tissue. But this did not happen though chromosome doubling is observed in root tissues of this form. If any doubling took place in aerial parts, the haploid tissue may have outgrown it. Fertility in the sterile haploid would be too easily recognized to be overlooked, since the doubled haploid is highly self-fertile. The conclusion is therefore evident that the tomato haploid is exceedingly stable tissue.

Parthenocarpy

Parthenocarpic development of haploid fruits is rare, although at certain seasons and particularly when the plants are in poor condition, one or two small, seedless fruits form. A total of 275 parthenocarpic fruits were examined and recorded. The very great majority of these contained nothing but abortive seeds. From open-pollinated fruits, only a dozen seeds were found. These on test proved to be either typical diploids or F_1 plants of outcrosses with other varieties. There was no increase or decrease of parthenocarpic fruiting during the experimental period.

Mutation

Particular attention was given to the discovery of localized mutation tissue in leaves, stems, and inflorescence, since any monogenic change in the haploid set of genes should be immediately apparent if the mutant area were of sufficient size to be visible. Search was made especially for chlorophyll mutations. But no indisputable cases were recorded. Occasionally small areas of yellow or pale green tissue were noted but all these seemed to be merely local disturbances, largely of virus or insect infection. No large variant sectors were observed in chlorophyll characters.

Only one clear case of variation was noted. This was a single leaf variation similar to the known "wiry" mutation, a recessive monogenic character. Unfortunately the axillary sprout of this leaf was cut out in the normal greenhouse procedure of training plants to a single stem.

Virus infection

During the experimental period the haploid suffered two major epidemics of mosaic infection plus a third minor attack. The two earlier epidemics (1930 and 1935) were serious enough to render continuation of the haploid stocks very uncertain. Precautions were taken to select disease-free cuttings. Eventually the infection subsided.

The virus in the 1930 and 1935 epidemics came from our regular genetic stocks of diploid tomato plants which also were infected at these times. Since we were not working on the virus problem then, no data are available concerning the virus strains involved in these natural epidemics. In 1936 a mild epidemic arose, but constant selection of disease-free cuttings held it in check.

In 1937, when DR. J. W. GOWEN commenced his tobacco virus studies in the same greenhouse, various strains of the tobacco virus and appropriate test-plant species were available. On test, the haploid was found to carry the aucuba strain, masked, however, to such an extent that a careful observer could detect little or no infection.

Since 1935–1936, then, the haploid seems to have developed an immunity to the natural infection which was constantly affecting other tomato varieties in the same greenhouse. These observations are being investigated further by an assistant.

Diploid from Haploid

Earlier genetical and cytological work (LINDSTROM and KOOS 1931) has demonstrated the uniformity of the absolutely homozygous diploid derived from the haploid by asexual means. The coefficients of variability in fruit characters of size and shape are as low as seven to ten percent, one character (polar diameter of fruit) going as low as three percent. This diploid is highly self-fertile, with fruits averaging 40 to 100 seeds per fruit. Genetically it is homozygous for the known genes *d p O S R V C Sp Ri* A_1 *L U h Wt* W_1 W_2 and theoretically for all other genes, providing its origin from the haploid is recent enough to prevent the introduction of mutant genes.

Monogenic mutations

During a period from 1929, when the first doubled haploids were produced artificially, to the present time, fully 10,000 seedlings and mature plants of this form were under observation. Only one distinct variation was observed. This was a mutation to a recessive, "wiry" form, a monogenic change. This "wiry" is similar phenotypically to the forms reported earlier by LESLEY and LESLEY (1928) and by SCHIEMANN (1932) in which leaves, sepals, and petals are reduced largely to midrib, giving a wiry or thread-like effect to the plant. So reduced is the plant that it is sterile. Surprisingly this new "wiry," which is more extreme than the older mutants, depends on another gene, called now w_2. Crosses with the original w_1 of MISS SCHIEMANN'S type, called *Solanum filiforme* (through heterozygous parents) gave normal F_1 and an F_2 from diheterozygous normals approximating 9 normal: 7 wiry. The latter class always includes a few very extreme forms, probably the double recessives.

Two other mutations, one to a sub-dwarf and the other to a bluish-green tall, were observed, but they involve sterility and presumably are chromosomal aberrations.

Selection experiments in the diploid

The stability of the absolutely homozygous diploid was tested by the rigorous practice of discarding the best seedlings in each generation, saving

only the runts. In this test large numbers of 2n seedlings were grown in greenhouse flats (usually six standard flats, with six rows of 30 seedlings per row per generation) and, beginning at the two-week stage, the tallest and best were pulled in numbers to leave sufficient individual space in the row so that there was no overcrowding as the plants grew. This selection was continued for six to eight weeks until only two to three plants were left in each flat. These were then further selected on the basis of plant

TABLE 1

Analysis of variance of tomato seedling green weights of three selection lines (A, B, C) and three control lines (H) after six generations of selection.

		DF	*SS*	VARIANCE
T_1	Total	71	1.1350	
	Blocks	3	.3783	
	Treatment	1	.0089	.0089
	Lines	2	.0058	.0029
	T×L	2	.0536	.0268
	Error	63	.6883	.0109
T_2	Total	71	10.149	
	Blocks	3	2.382	
	Treatment	1	.151	.151
	Lines	2	.323	.162
	T×L	2	.443	.222
	Error	63	6.850	.109
T_3	Total	71	93.893	
	Blocks	3	3.673	
	Treatment	1	5.336	5.336*
	Lines	2	.881	.441
	T×L	2	.991	.496
	Error	63	83.012	1.318

Treatment = controls vs. selection lines. Lines = three strains within each treatment. T_1 = three weeks, T_2 = four weeks, T_3 = five weeks age.

* F value = 4.0 (significant at five percent level).

size, put in eight-inch pots, and spaced separately in the greenhouse to avoid cross-pollination.

After the second generation of selection, three selection lines (A, B, and C) were maintained in duplicate from then on. Within each line one or two runts eventually were saved (except for reserves) to carry on the line, so that within each line one plant was saved from a total of approximately 400 seedlings or, lumping the three lines, three were saved from more than 1200 seedlings per generation. At the six week stage, the selected runts were often less than one fourth the size of the controls. Despite the extreme

care in planting to a uniform depth, it was probable that some of the differences in seedling size were due to accidents of planting; more, however, were due to variations in time of germination. During all this time, search was made for mutants of a retrogressive nature. None arose in the selection lines. The "wiry" mutant described earlier came in the control line (called H in this report).

These three selection lines were tested against the control after every third generation of selection. At the third and sixth generations, tests were

TABLE 2

Mean green weights per plant (grams) of tomato seedlings in three selection lines (A, B, C) and control (H) after six generations of selection.

	A	B	C	MEAN OF ABC	H
T_1 (3 weeks)	.44	.40	.45	.43	.45
T_2 (4 weeks)	1.35	1.02	1.31	1.21	1.31
T_3 (5 weeks)	3.10	2.90	3.02	3.01	3.55

made only on plant size (dry and green weight of tops) at three, four and five weeks of age, using randomized blocks. After the ninth generation of selection, fruit size was included in the tests. The controls in each case were doubled haploids, removed not more than two seed generations from the haploid itself to reduce any possible accumulation of undetected mutations.

No statistically significant differences in young plant size were evident after three generations of selection. The analysis of variance of plant weight after six generations of selection is shown in table 1. Samples were taken of three-, four-, and five-week old seedlings, using green weight of the tops. There were four replications of three selection lines and three control lines (the two treatments of table 1), with three plants per plot for T_1 (three weeks), two plants for T_2 (four weeks), and single plants for T_3 (five weeks). Triplicate samples of each line were taken at random.

There were no indications of differences due to selection at the three- or four-week stage. But at the five-week stage, there was a barely significant (five percent level) difference in the lines. As may be noted in table 2, all the selection lines showed a slightly lower mean seedling weight than did the control. Environmental variability at this stage, however, was great enough to render these mean differences of doubtful significance, a fact corroborated by later experiments.

The 1939 fruit measurement data on the three selection lines (A, B, C)

TABLE 3

Analysis of variance of tomato fruit weights in ninth selection generation in three lines and control. 1939 test.

	DF	*SS*	VARIANCE
Total	23	514.01	
Replications	5	106.51	
Lines	3	196.17	65.39*
L×R	15	211.33	14.09

* F value=4.64 (F at .01=5.42)
Control—A line=46.0−43.6=−2.4±2.17 grams
Control—B line=46.0−46.2=0.2
Control—C line=46.0−51.4=5.4±2.17

and control (H) after nine generations are shown in table 3. Five fruits of each of 144 plants (four lines×six plants per plot×six replications) were weighed and measured for polar and equatorial diameter of fruit. Only fruit weight data are treated here, since the correlations involving polar and equatorial diameters and weight are so high as to make a report of these calculations unnecessary (LINDSTROM 1926).

The variance for lines is significant only at the five percent point, indi-

TABLE 4

Analysis of variance of three selection generations and four lines. Tomato fruit weights. 1940 test.

	DF	*SS*	*Variance*
Total	119	1909.59	
Replications	9	250.34	
Lines	3	65.96	21.99
Generations	2	144.07	72.04**
L×G	6	241.66	40.28
Error	99	1207.56	12.20

10 replications of three-plant plots, with five measurements per plant.
** Highly significant
F value of Lines=1.8 (0.5 level=2.7)
F value for treatments (L, G, and L×G) at 11 DF=3.4 (0.1 level=2.4)

cating very little effect of selection on fruit weight. When the C line mean is compared with the control, a small but significant difference is manifest. Curiously the C line has a larger mean fruit weight than the control, despite the fact that selection was in the direction of a smaller plant. A plausible explanation would be that the selection process in the C line involved a slower-growing mutant which in turn proved to be a later, larger-

TABLE 5

Means of fruit weights (grams). 1940 test.

LINES	3RD GENERATION	6TH GENERATION	9TH GENERATION	MEAN
Control	43.0	37.3	41.5	40.6
A	38.6	39.9	37.1	38.5
B	41.0	37.5	39.4	39.3
C	39.3	37.0	41.7	39.3
Mean	40.2	37.9	39.9	39.4

fruited type. This possibility was tested more critically in 1940 with a larger experiment involving ten replications of the four lines and including the third, sixth, and ninth generations of selection.

In table 4 appear results of the 1940 experiment on fruit weight measurements of four lines (A, B, C, and control H), three generations of selection (third, sixth, and ninth), and ten replications of three-plant plots in randomized blocks. Five fruits of each plant were measured and weighed. In this experiment there is no verification of the 1939 test, the variance for fruit weight of lines being wholly non-significant.

The 1940 results may also be evaluated in table 5 giving the mean fruit weights. Line C is not significantly greater than the control in the ninth generation. Lines A and B are smaller but not significantly so. An analysis

TABLE 6

*Analysis of variance of tomato seedling dry weight at six weeks with four lines and three generations of selection.**

	DF	*SS*	VARIANCE
Total	68	0.4612	
Replications	5	0.0179	0.0036
Treatment	11	0.0671	0.0061
Error	52	0.3762	0.0072

* Three missing plots.
Treatment = Lines, Generations and L×G.

by generations shows no trend that might be attributed to selection. The relatively high variance for generations seems to be a random affair. The only other known variable is age of seed which, in this case, is known not to have caused any differential germination or seedling effect. We can conclude only that nine generations of selection to "break" this homozygous line have been unsuccessful as measured by fruit size.

The same material was also tested by obtaining dry weights of seedlings at six weeks of age. The data in table 6 are based on the same four lines, the same three generations of selection, with six replications of ten seedlings per plot. In this analysis, neither lines, generations, nor interaction exhibit any significant variation.

The mean dry weights per seedling from the experiment analyzed in table 6 are shown in table 7. While the analysis of variance showed no significant difference among the four lines, it is evident in table 7 that the means of all the selection lines are lower than that of the control (H). Evi-

TABLE 7

Mean dry weights per seedling in four lines and three selection generations (3rd, 6th, and 9th generation).

LINES	3	6	9	MEAN
H (Control)	.66	.62	.62	.630
A	.62	.60	.63	.616
B	.60	.60	.69	.628
C	.58	.57	.60	.582
Mean (grams)	.614	.595	.633	.614

dently the selection process produced a slight effect, but not enough to be statistically significant. A reasonable conclusion would be that the tomato genotype of the completely homozygous diploid from the haploid is relatively stable in the face of what seems to be a severe form of selection.

When this material was gathered, reserve seedlings were left in the plots for a determination of relative maturity as measured by time of blooming of the first cluster. The lines did not differ in this respect.

Tetraploid from Haploid

Since 1930, the homozygous autotetraploid form derived asexually from diploid, via the haploid, has been under observation and test. In the longest self-pollinated series, eight successive generations were produced. The main objective of this experiment was to test the stability and fertility of this form, since it represents the ultimate in genetic purity among tetraploids. Future attempts to use autotetraploids commercially must take into consideration the effect of continued selection for purity and hence homozygosity on fertility, particularly in seed-reproducing species.

With the unusually high initial sterility of this form, the problem automatically was resolved into a severe selection for fertility if for no other reason than merely to carry on the lines. Whereas the parental diploid produced 40 to 100 seeds per fruit, this autotetraploid rarely showed more than ten seeds per fruit. Fruit setting under natural conditions was exceedingly sparse. Whereas the diploid normally set four to seven fruits per

cluster, the 4n form set rarely one or two. The tetraploid was also more sensitive to environmental agencies affecting pollination and seed set. For example, during winter months when the diploid set a fair number of fruits, the 4n form was almost completely sterile, and the average number of seeds per fruit fell below ten. Under no circumstances did a single tetraploid plant ever produce more than four or five fruits, even when grown in the field where the diploid would yield more than 50 fruits.

Since the beginning of this 4n experiment, every fruit was harvested, every seed saved and planted except for a small reserve. Germination of this tetraploid seed was usually less than 70 percent. The progeny were uniformly 4n, but some of the seedlings died of vegetative abnormalities usually affecting the growing point. Chromosome counts were made often in the early stages of the experiment, but later the evaluation of the 4n condition was determined by the experienced eye. Search was made for parthenogenetic diploids, but not one appeared in more than 600 tetraploids carried to sexual maturity.

Selection for fertility during the eight consecutive generations revealed no increase. More than 400 plants in this series were tested. These were distributed over the consecutive selection generations as follows: first generation (7 plants), second (46), third (49), fourth (109), fifth (103) sixth (76), seventh (9), and eighth (16).

At stages in this experiment it was often difficult to obtain enough seed to carry on the selection lines. Quantitative data on number of fruits set were available, but environmental factors, especially those causing poor fruit set in winter months, made such data uncertain. Even under good greenhouse conditions and with the usual technique of daily tapping the plant to induce self-pollination, nearly half the plants were completely sterile or at most set one or two small fruits.

Within the limitations of this experiment where numbers were automatically limited by a high degree of sterility, there was no evidence of any increase in fertility in a genetically pure autotetraploid after eight generations of selection. During the period of this autotetraploid investigation, numerous allotetraploid tomatoes (largely *L. pimpinellifolium* × *L. esculentum*) were grown under the same conditions and in the same greenhouse. These consistently exhibited a much higher degree of fertility; therefore, the sterility of the autotetraploid forms cannot be attributed to environmental conditions.

During the eight generations, no marked variations in phenotypic characters of the autotetraploid series were observed. This is particularly true of mature plant and fruit characters. Certain vegetative abnormalities in the seedling stage are always apparent, but most of these affect the growing tips and either prove lethal or are outgrown.

DISCUSSION

Fourteen years of continuous asexual growth of one haploid genotype, nine generations of selection to "break" the homozygous diploid derived from the haploid, and eight generations of selection for fertility in the relatively sterile, homozygous autotetraploid derived from the original haploid all agree in demonstrating an exceedingly high degree of stability of the tomato genotype. The constancy of the haploid would seem to indicate an inherent stability of tomato germ plasm, a point not inconsistent with the relative infrequency of mutants in this species. Even chlorophyll mutations, common in most plant species, are rare in the tomato.

Following is a reasonably complete list of all reported monogenic variants:

Chromosome 1—dwarf, peach, ovate fruit, Wooly, compound fruit cluster, beaked fruit, locule number, rolled cotyledons; chromosome 2—yellow fruit flesh color; chromosome 3—brachytic, colorless fruit epidermis; chromosome 4—potato leaf, self-pruning (determinate growth), ridged leaves; chromosome 5—fasciated, Anthocyanin 1, jointless pedicel, leafy; chromosome 6—yellow (luteus) foliage; chromosome 7—uniform fruit ripening, Hairy, tangerine fruit color; chromosome 8—Anthocyanin 2; chromosome 9—dwarf 2; chromosome 10—wilty leaf, nippled fruit. This total of 26 genes is so small in relation to the number of pedigree cultures under observation by competent investigators that it also argues for a high stability of the tomato genotype.

Bearing on the commercial utilization of tetraploids in plant improvement programs, the experiment with the autotetraploid tomato strain affords some clue as to future possibilities with species reproducing by seed. The problem is one of what to expect in the fertility of a tetraploid with constant breeding or selection for purity or homozygosity. The autotetraploid tomato is one example of the ultimate in total homozygosity. Its high degree of sterility and the continuance of this sterility for eight generations of severe selection for fertility indicate an end point of sterility following continuous selection to purify a tetraploid genetically. In theory, such a selection program would seem to enhance the quadrivalent association of the chromosome sets with its attendant sterility. The tomato of course, may be a special case, and generalizations should be avoided, but at least it affords one definite experimental source of information on this point in a 4n plant breeding program.

SUMMARY

Observations and tests on a tomato haploid grown asexually for 14 years demonstrate a surprising stability of the genotype. No diploid sectors resulting in fertility arose. No large chlorophyll sectors or mutants were

observed. Only one somatic mutant was recorded, a presumptive mutant to the "wiry" condition. The haploid developed a form of natural immunity to virus infection, surviving two major epidemics, and now shows a masked type of aucuba infection.

The absolutely homozygous diploid derived asexually from the sterile haploid is a highly fertile form. Selection experiments for nine generations to "break" this pure genotype were relatively ineffective. One new, recessive, monogenic variant arose in the control (unselected) line. This was a new "wiry" (w_2) mutant, genetically different from the original "wiry."

In the absolutely homozygous autotetraploid derived originally from the haploid via the diploid, selection experiments for increased fertility in this highly sterile form were of no avail during eight generations, involving 410 plants. The bearing of this experiment on breeding plans to select for uniformity and homozygosity in tetraploids reproducing by seed is pointed out.

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VARIATION, ISOLATING MECHANISMS, AND HYBRIDIZATION IN CERTAIN TOADS[1]

ALBERT P. BLAIR[2]
Columbia University, New York

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INTRODUCTION

On the species problem the interests of genetics, taxonomy, and ecology converge to a remarkable extent, and it seems that only through coordination of the methods of these several disciplines can satisfactory progress in this field be made. Where closely-related species, or populations very nearly attaining the rank of species, are involved, the problem attains a maximum of complexity; it is just such cases, however, which appear most amenable to intensive investigation. The present article reports the results of an attempt to analyze the situation in a group of species of toads inhabiting the eastern United States.

MATERIAL AND TECHNIQUE

The collecting of approximately 4300 adult and 10,000 immature toads and tadpoles involved field work in Manitoba, North Dakota, South Dakota, Minnesota, Iowa, Missouri, Kansas, Oklahoma, Arkansas, Texas, Louisiana, Mississippi, Alabama, Georgia, South Carolina, North Carolina, Tennessee, Kentucky, Indiana, and Illinois. In addition 660 toads from three other sources were examined: (1) the University of Michigan Museum of Zoology, (2) other workers in Michigan, Pennsylvania, New York, Georgia, Arkansas, and Oklahoma, and (3) biological supply dealers in Wisconsin and North Carolina. In all, about 5000 adult toads from 39 states were critically examined and measured for various characters.

Four measurements were made on each toad: body length, foot length, parotoid gland width, and parotoid gland length. Body length was measured from the tip of the snout to the most posterior part of the body immediately behind the anus. Foot size was determined by flexing the tibiotarsal joint of the right foot over a rigid right-angled surface and measuring from the joint to the tip of the longest toe. The broadest and the longest dimensions of the right parotoid gland were measured for the characters parotoid width and parotoid length, respectively. Some error was undoubtedly present due to the fact that semi-rigid structures were concerned in the measurements, but remeasurement of several series showed that it was possible to reproduce data accurately. For instance, remeasurement of

[1] Submitted to the Faculty of the Graduate School of INDIANA UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Fellow of the National Research Council, 1940–41.

body length for 100 toads showed an average difference of measurement of 0.61 mm. In addition to the measurements, judgments were recorded on (1) whether or not the color of the parotoid glands was the same as that of the dorsal surface of the body, (2) whether or not dorsal spots were present, and (3) the extent of ventral spotting.

Toads collected in the breeding ponds were crossed in the laboratory without artificial stimulus in 23 matings. The clasping reaction is nonspecific, and no difficulty was encountered in inducing males collected during the breeding season to clasp females of the same or of different species. Because of differences in breeding seasons and the geographical separation of populations, it was sometimes impossible to mate toads without pituitary stimulation of one or both of the animals concerned. Both male and female were stimulated in seven crosses, the female alone was treated in 15 matings, and the male alone was injected in two crosses. Tadpoles were reared in tap water in shallow enamel pans containing Elodea and Cabomba; additional food was added in the form of dog biscuit, boiled egg yolk, and peanut butter. Young toads were fed on aphids and vestigial-winged *Drosophila melanogaster* until they were large enough to take pieces of earthworm.

FIGURE 1 (left).—Approximate ranges of *Bufo americanus* (A), *B. terrestris* (T), and *B. valliceps* (V). Body-foot ratio values are shown.

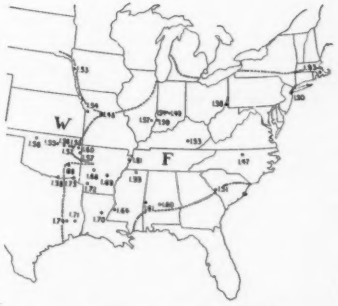

FIGURE 2 (right).—Approximate ranges of *Bufo fowleri* (F), and *B. woodhousii* (W). Body-foot ratio values are shown.

THE *americanus-terrestris-fowleri-woodhousii* COMPLEX

The toads found in the United States east of the plains may be listed as follows (see figs. 1 and 2 for approximate ranges): *Bufo quercicus*, found in the southeastern part of the United States; *B. terrestris*, occupying essentially an identical range with the preceding form; *B. fowleri*, occupying most of the eastern half of the United States; *B. americanus*, with a range

in large part duplicating that of *B. fowleri* but extending farther north and west and not so far south; and *B. valliceps*, found in parts of Texas and Louisiana. At the limits of their western ranges *B. fowleri* and *B. americanus* come in contact with *B. woodhousii*.

Bufo quercicus is a minute animal (under 30 mm) and cannot be confused with other adult toads; its phylogenetic relationships are obscure. *Bufo valliceps*, the northernmost representative of a Central American phylogenetic series, is distinguished by its triangular parotoid glands and the strong development of its cranial crests. Large knob-like swellings on the posterior ends of the supraorbital cranial crests are considered diagnostic for *B. terrestris*. The remaining three forms—*B. americanus*, *B. fowleri*, and *B. woodhousii*—are less certainly distinguished. What is usually considered typical *B. americanus* is a toad that is spotted ventrally, tends toward a reddish coloration, is quite spiny, has reniform parotoid glands with

TABLE 1

Local variation in Bufo fowleri, B. terrestris, and B. valliceps.

LOCALITY	NO. SPEC.	BODY	FOOT	BODY/FOOT	PAROTOID LENGTH	PAROTOID WIDTH	PAROTOID LENGTH/WIDTH
			Bufo fowleri				
Lakeville	30	55.250± .781	35.950± .524	1.531± .012	10.483± .227	5.776± .125	2.079± .036
Staten Island	7	58.522± .369	39.092± .594	1.501± .023	11.914± .240	6.007± .136	1.985± .048
Wheeling	11	51.586± .763	32.496± .816	1.581± .019	10.154± .197	5.114± .187	2.018± .069
Bloomington	554	58.370± .177	37.960± .126	1.583± .003	11.982± .052	5.830± .028	2.061± .008
Nashville	115	56.564± .434	37.880± .312	1.493± .004	12.004± .104	5.800± .042	2.082± .019
Vincennes	34	57.302± .429	36.010± .351	1.591± .012	11.979± .158	5.478± .099	2.200± .037
Olney	15	60.882± .593	38.750± .454	1.570± .016	12.933± .266	6.030± .161	2.149± .062
Mt. Vernon	6	59.616± 1.585	38.950± 1.121	1.535± .025	12.283± .300	5.688± .553	2.365± .314
Elon College	25	58.750± .543	39.950± .357	1.467± .008	12.080± .208	6.318± .125	1.911± .041
Modoc	15	54.350± .983	35.816± .666	1.514± .012	11.300± .195	5.256± .171	2.225± .058
Benton	204	53.250± .234	33.410± .183	1.599± .006	10.420± .076	5.140± .064	2.160± .019
York	23	53.690± .606	33.342± .376	1.612± .013	10.895± .227	5.615± .115	1.951± .043
Holly Spgs.	38	54.372± .466	35.002± .327	1.554± .010	11.266± .159	5.760± .110	1.974± .034
Vicksburg	11	55.768± .653	34.132± .870	1.644± .022	11.745± .268	5.664± .163	2.072± .045
Osceola	8	61.400± 2.250	38.200± .881	1.615± .031	12.950± .453	5.655± .164	1.940± .042
Pine Bluff	8	57.700± .590	33.950± .655	1.690± .024	10.075± .430	5.400± .272	1.875± .070
Hot Springs	7	55.378± 2.165	33.092± 1.449	1.681± .043	10.843± .569	5.778± .332	1.891± .043
Fulton	16	53.573± 1.078	31.450± .820	1.722± .019	10.575± .227	5.300± .169	2.026± .057
Scraper	169	58.588± .322	36.696± .234	1.600± .004	11.939± .092	5.450± .056	2.211± .019
Stilwell	8	60.700± 1.601	38.950± 1.069	1.574± .014	12.512± .574	5.350± .320	2.345± .088
Wilburton	48	55.158± .432	32.950± .333	1.665± .011	11.116± .146	5.942± .111	1.871± .023
Garvin	18	56.506± .644	32.726± .501	1.735± .015	11.008± .234	5.400± .272	2.052± .048
Jena	88	56.676± .562	33.176± .318	1.703± .008	11.149± .127	5.386± .082	2.083± .026
Woodvil'e	31	56.594± .533	32.970± .441	1.711± .016	11.361± .113	5.420± .118	2.170± .046
Pt. Blank	74	55.348± .475	31.676± .270	1.737± .010	10.970± .124	5.258± .072	2.115± .029
			Bufo terrestris				
Montezuma	6	55.950± 1.460	34.284± .802	1.620± .031	10.200± .182	5.616± .281	1.835± .094
Gulfport	7	55.092± 1.300	33.950± .873	1.638± .027	10.485± .420	5.264± .144	2.011± .082
			Bufo valliceps				
Pt. Blank	81	62.494± .479	35.900± .298	1.697± .019	9.070± .108	5.634± .067	1.609± .019

TABLE 2

Local variation in Bufo americanus and B. woodhousii.

LOCALITY	NO. SPEC.	BODY	FOOT	BODY/FOOT	PAROTOID LENGTH	PAROTOID WIDTH	PAROTOID LENGTH/WIDTH
			Bufo americanus				
Hyde Park	6	61.616±1.407	39.282± .211	1.555±.029	12.533±.543	8.416±.998	1.565±.115
Northampton	7	66.806±1.299	42.236±1.106	1.600±.042	11.985±.359	6.636±.286	1.848±.111
Staten Island	6	76.082±1.667	53.616±1.407	1.435±.029	14.783±.436	8.350±.565	1.805±.106
Polk	44	71.996± .884	45.810± .467	1.577±.012	13.153±.177	7.104±.111	1.866±.096
Pinckney	11	59.408±1.419	42.676±1.006	1.553±.025	11.291±.435	6.386±.181	1.780±.059
Oshkosh	55	62.822± .482	40.676± .195	1.565±.022	11.763±.128	6.966±.089	1.694±.022
Winona Lake	19	62.792± .564	41.528± .849	1.524±.023	12.489±.256	6.855±.233	1.852±.063
Bloomington	85	56.350± .467	36.892± .356	1.528±.007	11.700±.115	6.410±.062	1.882±.029
Nashville	59	54.560± .603	35.306± .362	1.546±.007	11.523±.136	6.100±.085	1.899±.024
Highlands	7	72.808±1.787	49.664±1.341	1.475±.026	14.128±.254	7.893±.312	1.779±.052
Princeton	13	61.950± .652	38.258± .151	1.616±.014	13.046±.148	8.012±.217	1.736±.044
Blue Springs	10	66.190±1.202	39.550± .737	1.666±.018	13.400±.333	7.310±.316	1.811±.054
Nevada	9	63.812±1.352	37.504± .205	1.706±.027	12.811±.431	7.372±.640	1.805±.145
Cleveland	9	59.060±2.003	35.282±1.001	1.673±.024	12.366±.571	6.799±.343	1.825±.050
Tulsa	23	57.602± .784	34.036± .583	1.693±.019	12.004±.234	6.228±.152	1.928±.043
Winslow	46	62.124± .624	37.810± .430	1.644±.011	13.004±.199	6.904±.117	1.870±.023
Imboden	11	58.858±1.347	32.676± .864	1.701±.014	12.018±.354	6.314±.319	1.979±.073
Wilburton	25	57.470± .825	34.750± .486	1.662±.019	11.140±.280	6.142±.192	1.827±.033
			Bufo woodhousii				
Onawa	19	75.740±1.375	49.528± .960	1.530±.015	14.647±.309	6.982±.200	2.105±.046
LaMine	30	62.350± .887	42.150± .613	1.484±.014	12.366±.198	6.963±.141	1.775±.024
Blue Springs	8	77.200±1.461	50.450± .732	1.539±.016	14.762±.546	7.500±.270	2.090±.055
Choteau	12	78.282±1.076	49.780± .601	1.575±.013	15.366±.365	6.955±.203	2.215±.081
Inola	15	80.482± .810	51.282± .644	1.576±.013	15.066±.213	6.995±.202	2.185±.065
Tulsa	169	80.162± .422	51.390± .281	1.560±.005	15.226±.109	6.890±.070	2.232±.019
Pawnee	9	81.282±1.491	52.636± .667	1.550±.022	15.255±.489	7.372±.377	2.065±.059
Waynoka	10	74.750±2.024	47.950±1.575	1.564±.012	14.650±.466	6.795±.364	2.081±.097
Yuba	7	66.806±1.739	42.236±1.016	1.583±.013	12.700±.598	6.069±.594	2.105±.056

a longitudinal crest connecting the parotoid gland with the postorbital crest, and breeds earlier than *B. fowleri* or *B. woodhousii*. *Bufo fowleri* and *B. woodhousii*, as ordinarily recognized, are relatively unspotted below, rarely tend toward a reddish coloration, are rarely spiny, have straight parotoid glands with no longitudinal crest connecting the gland with the postorbital crest, and breed later than *B. americanus*. *Bufo fowleri* and *B. woodhousii* seem best separated by size difference; few adult *B. fowleri* attain the minimum size at which *B. woodhousii* begins breeding. The herpetological literature of the last 80 years exhibits a remarkable diversity of opinion as to the relationships of the toads under consideration. No better demonstration of this uncertainty of opinion can be found than the multiplicity of combinations of specific and subspecific names which persists to the present day in the literature of the group.

VARIABILITY

Tables 1 and 2 give measurements for *B. fowleri* from 25 localities, *B. americanus* from 18 localities, *B. woodhousii* from nine localities, *B. terres-*

tris from two localities, and *B. valliceps* from one locality. Toads were classified as to species on general impression of the sum total of characters. In many localities where two or more species were present intermediate animals not readily referable to one species or another were found. Such animals are not included in tables 1 and 2. The following tabulation indicates the number of specimens considered from each locality and the number omitted as not readily referable to one species or another:

Locality	Classified	Unclassified	Locality	Classified	Unclassified
Hyde Park, Mass.	6	0	Nevada, Mo.	9	0
Northampton, Mass.	7	0	Waynoka, Okla.	10	0
Lakeville, Mass.	30	0	Pawnee, Okla.	9	0
Staten Island, N. Y.	13	0	Cleveland, Okla.	9	0
Polk, Pa.	44	0	Tulsa, Okla.	192	7
Wheeling, W. Va.	11	0	Inola, Okla.	15	0
Pinckney, Mich.	11	0	Choteau, Okla.	12	0
Oshkosh, Wis.	55	0	Scraper, Okla.	169	4
Winona Lake, Ind.	19	1	Stilwell, Okla.	8	1
Bloomington, Ind.	639	60	Wilburton, Okla.	73	0
Nashville, Ind.	174	22	Yuba, Okla.	7	0
Vincennes, Ind.	34	5	Garvin, Okla.	18	0
Olney, Ill.	15	5	Osceola, Ark.	8	0
Mt. Vernon, Ky.	6	1	Imboden, Ark.	11	0
Elon College, N. C.	25	0	Winslow, Ark.	46	0
Highlands, N. C.	7	0	Hot Springs, Ark.	7	0
Modoc, S. C.	15	0	Pine Bluff, Ark.	8	0
Montezuma, Ga.	6	0	Fulton, Ark.	16	0
Benton, Ala.	204	2	Holly Spgs., Miss.	38	0
York, Ala.	23	10	Vicksburg, Miss.	11	0
Onawa, Ia.	19	0	Gulfport, Miss.	7	0
Princeton, Mo.	13	0	Jena, La.	88	0
LaMine, Mo.	30	0	Woodville, Tex.	31	0
Blue Springs, Mo.	18	1	Pt. Blank, Tex.	155	5

The Mississippi valley localities of Osceola, Holly Springs, Pine Bluff, Hot Springs, Fulton, Garvin, Vicksburg, Jena, Woodville, and Point Blank are alike in that no *B. americanus* has been found at any of them. Nevertheless, specimens with some *americanus*-like characters appear in all of these populations. Such specimens have been included in the measurements of tables 1 and 2. Two other localities deserve particular mention. The majority of specimens from LaMine and Yuba, localities on the boundary line between *B. fowleri* and *B. woodhousii*, are referable to neither form and have been placed under *B. woodhousii*, since they perhaps resemble this form a little more than they do *B. fowleri*.

Measurements for body size, foot size, parotoid gland length, and parotoid gland width show no definite geographical gradients (clines). Parotoid

gland length-width ratio likewise seems to vary irregularly. However, body-foot ratio for *B. fowleri* shows a definite cline (fig. 1, 2), with the relatively smallest feet in the southwestern part of the range and larger feet toward the northeast. A similar cline is observed in *B. americanus*. Data for *B. woodhousii* are insufficient to speak positively concerning body-foot ratio variation, but it seems evident that the cline which is found in *B. fowleri* does not continue into the territory of *B. woodhousii*.

The judgments on presence or absence of spots, parotoid color, and extent of ventral spotting, the writer is aware, are inherently less accurate than measurements. It is felt, however, that such data may be used to advantage if the limitations are kept in mind. The percentage of animals in a given collection showing definite dorsal spots is given in the following tabulation:

B. fowleri		*B. americanus*	
Lakeville	80	Hyde Park	0
Staten Island	100	Northampton	28
Wheeling	100	Staten Island	67
Bloomington	96	Polk	93
Nashville	97	Pinckney	91
Vincennes	76	Oshkosh	100
Olney	27	Winona Lake	95
Mt. Vernon	100	Bloomington	61
Elon College	76	Nashville	64
Modoc	100	Highlands	43
Benton	98	Princeton	15
York	91	Blue Springs	50
Holly Springs	70	Nevada	20
Osceola	100	Cleveland	22
Vicksburg	18	Tulsa	56
Jena	58	Winslow	9
Pine Bluff	25	Imboden	27
Hot Springs	57		
Scraper	37	*B. woodhousii*	
Stilwell	37	Onawa	100
Wilburton	12	LaMine	87
Garvin	6	Blue Springs	58
Fulton	0	Choteau	66
Woodville	19	Inola	87
Point Blank	27	Tulsa	69
		Pawnee	33
		Waynoka	90
		Yuba	14

While there are some exceptional localities, there is indication in both *B. fowleri* and *B. americanus* of a cline similar to that for body-foot ratio. In general, populations in the southwestern part of the range show more unspotted specimens than populations farther northeast.

Parotoid color and ventral spotting data show no clinal tendencies but

may be looked upon as species differentials between *B. americanus* on the one hand and *B. fowleri* and *B. woodhousii* on the other hand. Forty-two percent of the *B. americanus* specimens have parotoid glands which are not of the same color as the back, while only 12 percent of the *B. fowleri* have parotoid glands showing such a color difference. In *B. fowleri* and *B. woodhousii* ventral spots, if any, are confined to the anterior half of the ventral surface; in *B. americanus* the ventral surface may vary from unspotted to heavily spotted over the entire ventral surface.

Extensive collecting data indicate that wherever two or more members of the *americanus-terrestris-fowleri-woodhousii* complex occur some intermediates are to be expected. Even the Point Blank series, in which the two toads involved are not closely related and are quite different in appearance, contains five intermediates in 155 specimens. Other localities present other conditions. Of 36 toads collected at York, Alabama, three were referred to *B. terrestris*, 23 to *B. fowleri*, and ten were called intermediates. The maximum frequency of intermediates is attained in the collections from LaMine and Yuba, where the entire collections seem best referred to as intermediate. The *B. fowleri* of the lower Mississippi Valley have been mentioned as somewhat different from other *B. fowleri*.

PHYSIOLOGICAL CHARACTERISTICS

In view of the indications of hybridization it seems desirable to consider the physiological characteristics which might aid in maintaining distinct species populations. The toad species under consideration possess physiological differences which are expressed in breeding call, breeding time, and breeding site. The droning mating calls of *B. fowleri* and *B. woodhousii* are extremely similar; that of *B. woodhousii* is louder, but this is to be expected in view of the difference in size. In contrast to the droning calls of *B. fowleri* and *B. woodhousii*, the calls of *B. americanus* and *B. terrestris* are trills, high-pitched in *B. americanus* and somewhat lower in *B. terrestris*. Critical investigation of call variation must be based upon accurate measurement with suitable physical apparatus; such a study has not yet been made. It is possible, however, to distinguish certain variations from the two call types—the drone and the trill—in the field. Calls best described as intermediate have been heard in a number of localities—Bloomington, Nashville, Mt. Vernon, Scraper, York, Benton, etc.—where two species call in the ponds at the same time. Field observations indicate that animals with intermediate calls are usually somewhat intermediate morphologically.

As a rule *B. americanus* breeds earlier than either *B. fowleri* or *B. woodhousii*. A collecting site south of Wilburton, Oklahoma, yielded only *B. americanus* on April 8, 1940, while on May 26, 1939, only *B. fowleri* were

taken at the same locality. At Bloomington, Indiana, the earliest dates for *B. americanus* and *B. fowleri* are as follows for 1938, 1939, and 1940:

	1938	1939	1940
B americanus	March 22	March 23	April 2
B. fowleri	April 13	April 17	April 30

Separation of breeding times for different species is far from complete, however, as the following records show:

Locality	Date	Species breeding
Cleveland, Okla.	June 15, 1937	*B. woodhousii, B. americanus*
Blue Springs, Mo.	June 17, 1938	*B. woodhousii, B. americanus*
Nashville, Ind.	April 26, 1938	*B. americanus, B. fowleri*
York, Ala.	June 3, 1939	*B. fowleri, B. terrestris*
Pt. Blank, Tex.	May 28, 1939	*B. fowleri, B. valliceps*

In spite of breeding at the same time, two species may not come in contact if they utilize different breeding sites. *Bufo woodhousii* and *B. americanus* collected June 15, 1937, at Cleveland, Oklahoma, were breeding in the same general locality, but the *B. americanus* were in shallow ditches while the *B. woodhousii* were in more permanent bodies of water. *Bufo fowleri* taken near Wilburton May 26, 1939, were all collected from a creek; *B. americanus* taken at the same locality April 8, 1940, were all breeding in shallow rain pools, and not a single toad was found along the creek which was only a few hundred feet from the pools. But just as breeding times are not completely separate for various species, neither are breeding sites. *Bufo woodhousii* and *B. americanus*, *B. fowleri* and *B. americanus*, *B. terrestris* and *B. fowleri*, and *B. fowleri* and *B. valliceps*—each of these combinations has been found breeding in the same body of water. However, in spite of intensive and extensive collecting along the line separating *B. fowleri* and *B. woodhousii*, the writer has never found unquestionable examples of these two forms in the same body of water.

VIABILITY AND FERTILITY OF EXPERIMENTAL HYBRIDS

Since variation data suggested the possibility of hybridization in nature, it was deemed advisable to investigate experimentally the possibility of gene exchange among the several forms involved. Two populations are effectively isolated from one another if mating fails to take place between members of the two groups, if mating occurs but fertilization is not effected, if fertilization takes place but the offspring are not viable, or if viable but sterile offspring are produced. The effectiveness of cross-fertilization and the viability of hybrid offspring were checked by the following crosses (see table 3):

2 ♀ *B. woodhousii* × ♂ *B. fowleri*
6 ♀ *B. fowleri* × ♂ *B. woodhousii*

TABLE 3

Laboratory hybridizations

NO.	FEMALE PARENT		MALE PARENT		DATE EGGS LAID	CLEAVAGE %	GASTRULATION %	LARVAE %	METAMORPHOSIS NUMBER
	SPECIES	SOURCE	SPECIES	SOURCE					
5	*Bufo fowleri*	Bloomington, Ind.	*B. woodhousii*	Mounds, Okla.	11/ 9/37	95	10	1	2
10	*B. fowleri*	Stilwell, Okla.	*B. woodhousii*	Choteau, Okla.	2/19/38	5	0	0	0
13	*B. fowleri*	Scraper, Okla.	*B. woodhousii*	Tulsa, Okla.	2/27/38	99	95	10	75
14	*B. woodhousii*	Choteau, Okla.	*B. americanus*	Bloomington, Ind.	3/25/38	+*	+*	+*	11
15	*B. woodhousii*	Tulsa, Okla.	*B. americanus*	Bloomington, Ind.	3/30/38	45	40	1	0
17	*B. americanus*	Bloomington, Ind.	*B. fowleri*	Bloomington, Ind.	4/17/38	95	+	+	100
19	*B. fowleri*	Nashville, Ind.	*B. americanus*	Nashville, Ind.	4/28/38	90	+	+	47
20	*B. fowleri*	Nashville, Ind.	*B. americanus*	Nashville, Ind.	4/28/38	80	+	+	42
21	*B. fowleri*	Nashville, Ind.	*B. americanus*	Nashville, Ind.	4/27/38	95	+	+	48
25	*B. woodhousii*	Tulsa, Okla.	*B. americanus*	Tulsa, Okla.	6/13/38	85	28	20	40
26	*B. fowleri*	Atlantic City, N. J.	*B. americanus*	Polk, Pa.	11/29/38	80	10	0	0
28	*B. woodhousii*	Owasso, Okla.	*B. fowleri*	Scraper, Okla.	2/18/39	90	5	3	34
30	*B. woodhousii*	Tulsa, Okla.	*B. americanus*	Bloomington, Ind.	3/29/39	95	90	80	175
31	*B. fowleri*	Point Blank, Tex.	*B. americanus*	Bloomington, Ind.	3/25/39	0	0	0	0
34	*B. woodhousii*	Owasso, Okla.	*B. fowleri*	Holly Spgs., Miss.	4/16/39	95	90	+	41
35	*B. fowleri*	Baton Rouge, La.	*B. americanus*	Bloomington, Ind.	4/24/39	97	+	+	47
36	*B. fowleri*	Baton Rouge, La.	*B. americanus*	Bloomington, Ind.	4/25/39	30	10	0	0
37	*B. fowleri*	Baton Rouge, La.	*B. americanus*	Bloomington, Ind.	4/25/39	60	+	+	20
39	*B. americanus*	Oshkosh, Wis.	*B. fowleri*	Baton Rouge, La.	4/26/39	90	+	+	37
41	*B. americanus*	Bloomington, Ind.	*B. fowleri*	Bloomington, Ind.	4/27/39	95	+	+	31
44	*B. fowleri*	Bloomington, Ind.	*B. woodhousii*	Yuba, Okla.	5/ 8/39	98	30	15	9
45	*B. fowleri*	Bloomington, Ind.	*B. woodhousii*	Laverne, Okla.	5/ 8/39	92	10	1	4
47	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Bloomington, Ind.	5/ 8/39	99	+	40	40
48	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Bloomington, Ind.	5/ 8/39	98	+	44	24
51	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Winona Lake, Ind.	5/ 8/39	1	0	0	0
56	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	95	+	80	42
57	*B. fowleri*	Bloomington, Ind.	*B. woodhousii*	Tulsa, Okla.	5/18/39	90	+	40	18
58	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	96	+	95	30
59	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	85	+	4	15
60	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	87	+	60	12
61	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	80	+	60	61
62	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	90	+	50	16
63	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Bloomington, Ind.	5/18/39	98	+	95	26
64	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Bloomington, Ind.	5/18/39	95	+	60	0
65	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	75	+	4	16
66	*B. fowleri*	Bloomington, Ind.	*B. americanus*	Tulsa, Okla.	5/18/39	80	+	4	7
67	*B. terrestris*	Silver Spgs., Fla.	*B. valiiceps*	Point Blank, Tex.	4/ 5/40	5	4	1	7
68	*B. americanus*	Oshkosh, Wis.	*B. terrestris*	Silver Spgs., Fla.	4/ 3/40	95	+	90	111
69	*B. americanus*	Demorest, Ga.	*B. terrestris*	Silver Spgs., Fla.	3/ 3/40	75	+	70	117
70	*B. terrestris*	Silver Spgs., Fla.	*B. fowleri*	Bloomington, Ind.	4/ 3/40	98	+	95	55
71	*B. terrestris*	Silver Spgs., Fla.	*B. americanus*	Bloomington, Ind.	4/ 3/40	98	+	95	48
72	*B. terrestris*	Silver Spgs., Fla.	*B. americanus*	Bloomington, Ind.	4/ 5/40	90	85	80	55
74	*B. americanus*	Sedalia, Mo.	*B. terrestris*	Silver Spgs., Fla.	4/13/40	90	+	+	16
75	*B. americanus*	Bloomington, Ind.	*B. terrestris*	Silver Spgs., Fla.	4/18/40	85	+	+	3
76	*B. americanus*	Bloomington, Ind.	*B. terrestris*	Silver Spgs., Fla.	4/16/40	95	+	+	5
77	*B. americanus*	Bloomington, Ind.	*B. terrestris*	Silver Spgs., Fla.	4/18/40	10	+	+	7
79	*B. americanus*	Oshkosh, Wis.	*B. woodhousii*	Dallas, Tex..	5/ 4/40	80	+	+	10

* The plus sign indicates that some reached this stage; exact data lacking.

4 ♀ *B. woodhousii* × ♂ *B. americanus*
1 ♀ *B. americanus* × ♂ *B. woodhousii*
3 ♀ *B. americanus* × ♂ *B. fowleri*
21 ♀ *B. fowleri* × ♂ *B. americanus*
2 ♀ *B. terrestris* × ♂ *B. americanus*
6 ♀ *B. americanus* × ♂ *B. terrestris*
1 ♀ *B. terrestris* × ♂ *B. fowleri*
1 ♀ *B. terrestris* × ♂ *B. valliceps*

Eggs were laid by female toads while being clasped by male toads in 47 instances, and in 46 of these cleavage was initiated. Forty-five of the 46 batches of eggs reached the yolk-plug stage. Three of these died in gastrulation, leaving 42 batches producing tadpoles. Only two of the 42 batches producing tadpoles failed to have tadpoles metamorphose. A total of 1504 tadpoles matamorphosed; however, this does not give a true picture of viability, since limitations of laboratory space necessitated the reduction of the progeny of most crosses to a very small fraction of their initial size. A single female toad lays from 3000 to 25,000 eggs, and the rearing of all the offspring of a single cross is a tremendous undertaking. Some developmental abnormalities were found, but it was not found possible to correlate these with any particular species crosses. Considerable difficulty was encountered in rearing toads from metamorphosis to adulthood; that this difficulty is not entirely due to their hybrid nature may be inferred from the fact that non-hybrid toads seem no less difficult to rear.

The hybrids, in general, are intermediate between the parental forms. On the whole, hybrids from crosses involving *B. americanus* look more like this species than the other parent. This is not always the case, however. A toad from hybridization No. 56 (♀ *B. fowleri* × ♂ *B. americanus*) has the olive dorsal coloring and small hind leg warts characteristic of *B. fowleri*. At first glance the toad might be taken for *B. fowleri*, but closer inspection reveals that the parotoid glands and ventral spotting are more nearly like those of *B. americanus*. While considerable variation may exist in a batch of F_1 toads, in only one case has there seemed to be a definite division into classes with regard to a given character. Of the offspring from hybridization No. 69 (♀ *B. americanus* × ♂ *B. terrestris*) about half of the individuals were bright reddish-brown and the remainder were olive gray. The color of the female in this cross was a peculiar reddish-brown, a color observed several times by the author in toads from the extreme southern Appalachian Mountains but never from any other locality. This result suggests the reddish-brown color to be due to a single dominant mutant gene. Offspring from crosses of *B. americanus* with *B. fowleri* from the lower Mississippi valley are more *americanus*-like than offspring from

crosses of *B. americanus* and *B. fowleri* from localities other than the lower Mississippi valley. In some respects the offspring of crosses involving *B. woodhousii* and lower Mississippi valley *B. fowleri* look more like *B. americanus* than either *B. woodhousii* or *B. fowleri*.

More important than the question of hybrid viability is that of hybrid fertility. Eight hybrid toads have developed the thumb pads and dark throats of mature males. The number of the cross, parents involved, and size and age in months at which secondary sex characters appeared are given in the following table:

No. 14	♀ *B. woodhousii* × ♂ *B. americanus*	51.2 mm	14½ months
No. 14	♀ *B. woodhousii* × ♂ *B. americanus*	55.8 mm	16 months
No. 14	♀ *B. woodhousii* × ♂ *B. americanus*	54.6 mm	17½ months
No. 19	♀ *B. fowleri* × ♂ *B. americanus*	53.4 mm	28 months
No. 21	♀ *B. fowleri* × ♂ *B. americanus*	58.0 mm	18 months
No. 34	♀ *B. woodhousii* × ♂ *B. fowleri*	45.7 mm	8½ months
No. 59	♀ *B. fowleri* × ♂ *B. americanus*	43.2 mm	17½ months
No. 61	♀ *B. fowleri* × ♂ *B. americanus*	44.0 mm	17 months

Two of the No. 14 hybrids and the No. 21 hybrid were backcrossed to female *B. americanus*. Results are summarized:

	Cleavage	Number Tadpoles	Number Metamorphosed
No. 14	90 percent	1424	24
No. 14	95 percent	1316	20
No. 21	80 percent	1820	49

Some developmental abnormalities were present, but the majority of tadpoles seemed normal. It must be recalled that the number of tadpoles metamorphosing does not give a true picture of viability. Since female toads show no secondary sex characters by which one can judge maturity, no crosses have been attempted with hybrid females. However, two female hybrids (♀ *B. woodhousii* × ♂ *B. fowleri*; ♀ *B. americanus* × ♂ *B. fowleri*) which died at the age of 32 months and 29 months, respectively, contained large masses of well-developed eggs.

THE BLOOMINGTON POPULATION

It seemed desirable to investigate in detail the interrelations of species populations which occur in the same localities, perhaps exchanging genic material to some extent, but nevertheless each maintaining its identity. Such situations are ideal for the study of isolating mechanisms.

A toad population in an area of approximately six square miles, located about three miles north of Bloomington, Indiana, was studied through three seasons. The principal breeding sites utilized by toads in the area were the Bloomington city reservoir and several flood plain sloughs and ponds about two miles down the creek valley from the reservoir. Some-

thing over 1900 toads, of which about 90 percent represent animals caught during the breeding season, were collected. Preliminary observations on the toads of the area revealed the presence of an early-breeding unit referable to *B. americanus* and a late-breeding unit referable to *B. fowleri*. More intensive investigation was undertaken to elucidate the relationships of these two groups. The top horizontal row of the histograms in figure 3 gives measurements for 950 male toads collected both in and out of breeding season from the fall of 1937 through the spring of 1940. Body length and foot length were measured as previously described. The shortest dis-

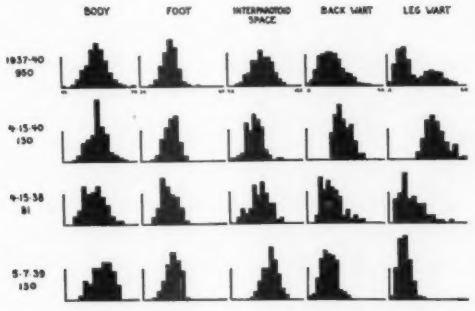

FIGURE 3.—Character variation in Bloomington male toads.

tance between the parotoid glands was taken as the interparotoid distance. What appeared at visual inspection as the largest dorsal wart was measured for the character back wart, and similarly the largest tibial wart was taken for the character leg wart. The histograms for body length, foot length, and interparotoid space show symmetrical distribution with little indication of other than a homogeneous population. The histogram for back wart size shows a decided skewness. A definitely bimodal distribution is evident for the character hind leg wart. If breeding time is considered, other differences are evident. The second, third, and fourth horizontal rows of the histograms in figure 3 give measurements for an early-breeding unit, an intermediate-breeding unit, and a late-breeding unit. Back wart size and interparotoid distance, as well as hind leg wart size, differ significantly for the three dates.

Since hind leg wart size showed the most distinct separation of the entire population into two units, its variation was followed carefully. Male toads were collected every night on which they were calling from April 3 to May 7, 1940. The row of histograms (fig. 4) entitled "males" gives hind leg wart variation for the various collecting dates. The area of each histogram is

roughly proportional to the number of toads calling on that date. The vertical dotted line on each histogram corresponds to the low point of the bimodal figure for hind leg wart size of 950 male toads in figure 3. It is evident that early in the breeding season all toads in the ponds are large-warted toads (*B. americanus*), that this condition gives way to one in which both large-warted and small-warted toads are present, and that finally the majority of toads in the ponds are small-warted (*B. fowleri*). The data for May 4 are of interest. The majority of the toads calling on

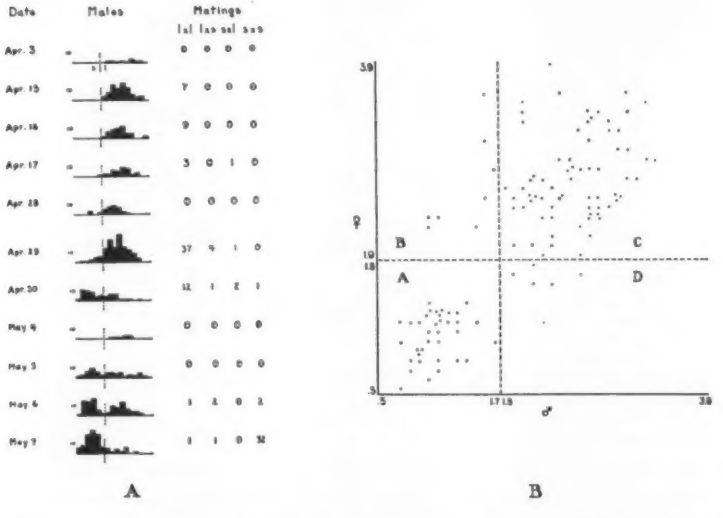

FIGURE 4.—A. Variation in hind leg wart size of male toads and in matings from April 3 to May 7, 1940, in the Bloomington toad population. B. Correlation of hind leg wart size of mated toads.

April 30 were small-warted, but after a cold interval only large-warted toads were calling on the cool night of May 4.

Since the breeding calls of *B. americanus* and *B. fowleri* are quite different, close attention was paid to calls during the period from April 3 to May 7. The few toads calling April 3 all had the high-pitched trill typical of *B. americanus*. Cold weather stopped all calling until April 15. Strong choruses of high-pitched trills were heard on this and the following two nights. Again cold weather intervened, and no more calls were heard until April 28. On this night it was at once apparent that the voices of many of the toads calling were huskier and had a less pronounced trill than the voices of the toads calling previously. Similar voices were heard the next

night. On the following night, April 30, there was a strong chorus composed of trills, calls of the variable husky type, and a new note, the droning call of *B. fowleri*. Cold weather stopped all calling for three days, but on the cool night of May 4 several toads were trilling. The next night trills, ranging from clear and high-pitched to husky and indistinct, and droning were heard. The droning call was in the majority by the following night, May 6, and by the next night it far outnumbered trills.

The data on male toad variability presented thus far indicate (1) that there is an early-breeding toad population (*B. americanus*) and a late-breeding population (*B. fowleri*) and (2) that the breeding seasons of the two overlap. What are the opportunities for exchange of genic material in this period of overlap? To a certain extent *B. americanus* and *B. fowleri* utilize different breeding sites. Temporary rain pools which are never used by *B. fowleri* may be used for breeding by *B. americanus*. The isolating mechanism is far from perfect, however. Even within a given pond or lake there may be concentrations of breeding *B. americanus* at certain points and concentrations of *B. fowleri* at other points. Here again the separation is incomplete; the author has collected the two species side by side on many occasions. There seems to be little specificity of the clasping reflex. Male toads readily clasp female toads of other species and inanimate objects as well. Evidence accumulated to date indicates that *B. americanus*$\times$*B. fowleri* hybrids are viable and fertile.

When both species are in the breeding ponds at the same time it should be possible to find interspecific matings in nature. A diligent search was made during the period April 3 to May 7 for mated toads. Since hind leg wart size shows the greatest differentiation of early and late-breeding populations, it should be the best criterion of interspecific matings. Hind leg wart size for 117 pairs is shown in the correlation chart figure 4B. The horizontal axis represents size of hind leg wart of the male and the vertical axis the size of hind leg wart of the female, each dot then indicating the hind leg wart size of a female and of the male found clasping this female. The vertical dash line between 1.7 and 1.8 indicates the low point between the two peaks in the bimodal histogram for hind leg wart size of 950 male toads shown in figure 3. Since the hind leg wart size of female toads averages 0.1 mm larger than that of males, the horizontal dash line is placed between 1.8 and 1.9. Dots in rectangle A then represent matings of small-warted toads with small-warted toads, dots in rectangle C represent matings of large-warted toads with large-warted toads, dots in rectangle B represent matings of large-warted females with small-warted males, and dots in rectangle D represent matings of small-warted females with large-warted males. It is evident that about 12 percent of the matings are between large-wated females and small-warted males, or between small-

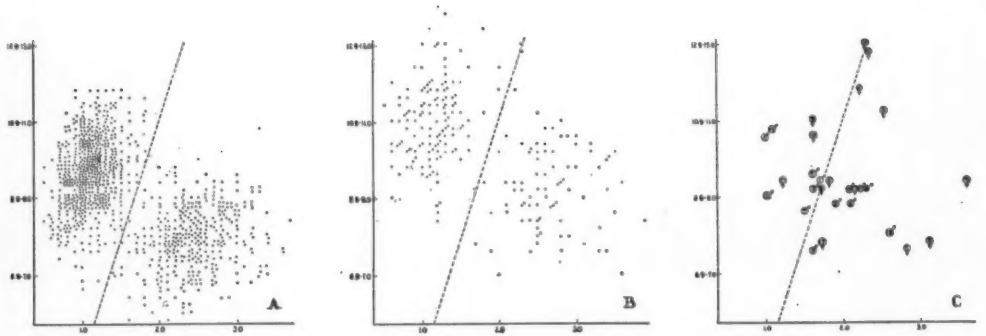

FIGURE 5.—A. Correlation of hind leg wart size and interparotoid space of Bloomington male toads. B. Same for Bloomington female toads. C. Correlation of hind leg wart size and interparotoid space of male and female toads from matings which suggest hybridization.

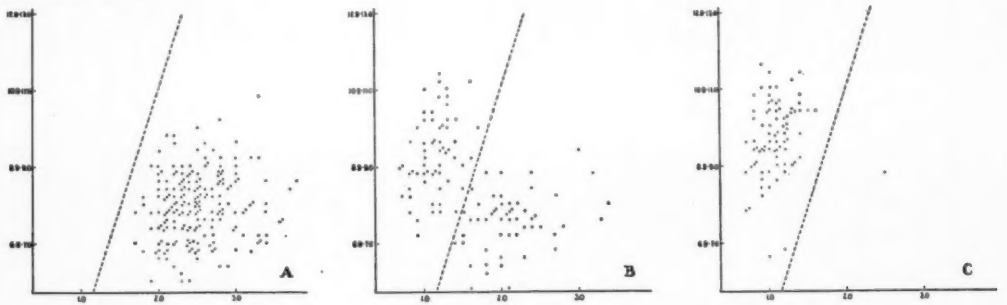

FIGURE 6.—A. Correlation of hind leg wart size and interparotoid space for toads collected early in the breeding season. B. Same for toads collected at intermediate breeding season. C. Same for toads collected late in the breeding season.

warted females and large-warted males. The temporal distribution of matings is shown in figure 4A under the title "matings."

What is the significance of matings between large-warted and small-warted toads? Is this hybridization? Or can it be explained on the basis of overlapping ranges of variation of the two species? Ten of the 14 examples are doubtful because of their proximity to the lines of demarcation between the two forms. The remaining four may be further examined. If hind leg wart size and interparotoid distance of male toads are graphed on a correlation basis (fig. 5A), the division into two units is more pronounced than on the basis of hind leg wart size alone. These two units can be divided by a more or less definite line. The same line serves to divide population units for similar data for females (fig. 5B). Let us now consider the four cases in question, on the basis of both hind leg wart and interparotoid distance (see fig. 5C). Three of the matings (Nos. 2, 12, and 14) involve male toads which are definitely *B. fowleri* and female toads which lie very near the line of demarcation between the two species. The fourth mating (No. 11) is a fairly clear-cut case of interspecific mating. Reexamination of the ten matings which were considered doubtful on the basis of hind leg wart alone reveals five matings (Nos. 1, 3, 4, 5, and 7) of female *B. americanus* with doubtful males, two matings (Nos. 6 and 13) of doubtful females with male *B. americanus*, one mating (No. 8) of a doubtful female with a doubtful male, and two matings (Nos. 9 and 10) which may be interspecific. In the fourteen matings, however, there is no case of mating of a toad far within the variation range of *B. americanus* with a toad far within the variation range of *B. fowleri*.

If hybridization between *B. americanus* and *B. fowleri* went on to any appreciable extent, one would expect the characters of *B. fowleri* to be eventually transmitted to the early-breeding toads and the characters of *B. americanus* to be transmitted to the late-breeding toads. If, however, hybridization should be of recent origin, or if recombination products are eliminated by natural selection, one might expect a relatively homogeneous early-breeding population, a heterogeneous mid-season population, and a relatively homogeneous late-breeding population. Figure 6 shows correlation of hind leg wart and interparotoid space for toads from three collecting dates, one early (fig. 6A), one late (fig. 6B), and one in mid-season (fig. 6C). It is apparent that there is less correlation of the two characters at the period of overlap. Similarly, observations on correlation of call and morphological characters at mid-season indicate that, while intermediate voices can frequently be traced down to morphologically intermediate toads, in some instances toads may have a high-pitched or husky trill and yet look predominantly like *B. fowleri*.

DISCUSSION

Two groups of conclusions, which are, in a sense, contradictory to each other, seem to follow from the data presented above. First, the toads inhabiting the eastern and central United States fall into four distinct arrays, which are designated as the species *Bufo americanus*, *B. terrestris*, *B. fowleri*, and *B. woodhousii*. Second, in most of the places where two or more of these species occur together, the populations contain some individuals which are intermediate between the species in question and which suggest that interspecific hybridization is taking place. Provided the intermediates are actually hybrids and provided they are about as viable and fertile as the parental forms, how can the discreteness of the species arrays themselves be maintained for any length of time?

Examination of morphological variation in *B. fowleri* (25 localities), *B. americanus* (18 localities), and *B. woodhousii* (nine localities) shows the populations of each species to be exactly alike in no two localities. Variation from locality to locality is haphazard for some characters, but body-foot ratio and frequency of dorsal spotting show geographical gradients (clines) from southwest to northeast. The gradients are parallel in *B. fowleri* and *B. americanus*, but a rather sharp break is observed where these species are supplanted by *B. woodhousii*. Individuals that are intermediate between, or show mixtures of characters of, different species may be interpreted either as extreme variants of the respective species or as hybrids between them. The first of these interpretations is rendered unlikely by the fact that the intermediates occur only in the regions where the distribution areas of the two putative parental species overlap. Thus, intermediates between *B. americanus* and *B. woodhousii* are confined to a strip of territory about 100 miles wide extending from eastern Oklahoma to eastern Nebraska (figs. 1, 2). The ranges of *B. fowleri* and *B. woodhousii* seem not to overlap; at the junction of their ranges the two species may be quite distinct or may be separated by an intermediate population. Intermediates between *B. fowleri* and *B. terrestris* are found in an area in the southeast where the distribution regions of these species overlap (figs. 1, 2). The areas of *B. fowleri* and *B. americanus* are in the main coterminous, and intermediates occur throughout. Moreover, as shown above, the intermediates found in nature are similar morphologically to known hybrids between the respective species obtained in laboratory experiments.

An unlimited exchange of genes among species populations residing in the same territory may be restricted or prevented altogether by a variety of isolating mechanisms. It is the presence of isolating mechanisms that permits the maintenance of species arrays as discrete entities. The author's observations suggest that such mechanisms may be operative in toad populations. *Bufo americanus* breeds earlier in the season than *B. fowleri*

or *B. woodhousii*. In a population at Bloomington, Indiana, which has been studied in detail in this respect, there exists an early-breeding unit (*B. americanus*) and a late-breeding unit (*B. fowleri*). However, the breeding periods of the two species overlap, and some of the individuals which reproduce during this intermediate period are morphologically intermediate between the two species. The seasonal isolation alone is thus insufficient to separate the species.

Difference in breeding sites (ecological isolation) is another mechanism that contributes to the separation of species. *Bufo americanus* sometimes breeds in shallow ponds which are never used by *B. fowleri* or *B. woodhousii*. At the junction of the ranges of *B. fowleri* and *B. woodhousii* in northeastern Oklahoma, the former breeds along creeks, while the latter utilizes for the most part prairie ponds. Where the east-west topographic change is abrupt, as the transition from Ozark Hills to Osage Plain, the ranges of the two species meet, but few or no intermediates are found. Where change is more gradual (LaMine and Yuba), intermediate populations are found. In mountainous regions of the Appalachians *B. americanus* reaches higher altitudes than *B. fowleri*. A third isolating mechanism, concerning which little is known but which may be extremely important, involves a form of sexual isolation. If toads respond to the mating calls of their own species, the opportunity for species crossing is greatly reduced. The writer is able to confirm the observation previously made by several workers that different species congregate in different parts of the same pond or other body of water. In the Bloomington population, however, individuals have been observed which possessed mating calls intermediate between *B. americanus* and *B. fowleri* and which were morphologically intermediate between the same species.

No isolating mechanism thus far detected can be regarded as sufficient to maintain the separation of the toad species under discussion. The scarcity of toad matings which are clearly interspecific indicates that a combination of several isolating mechanisms prevents the occurrence of mass hybridization. But, since these hybrids are fertile, there may nevertheless be an appreciable amount of exchange of genes among the species. This being the case, it is difficult to see how the identity of species can be maintained for a long time. A possible answer is that the hybridization is of relatively recent origin. Postglacial climatic changes and, more important still, human activities such as the clearing of forests and damming of streams may have so altered the ecological conditions as to affect the degree of isolation among toad species. If that is the case, the phenonena observed in these toads belong not to the field of the origin of new species but to that of the fusion and disintegration of old ones. Among plants such phenomena are not rare and appear to be engendered principally by human agencies

(ANDERSON and HUBRICHT 1938; RILEY 1939). On the average, isolating mechanisms maintaining the separation of animal species are more secure than those in plants. The toads may or may not prove to be an exceptional group in this respect.

SUMMARY

A morphological and statistical study of the toads inhabiting the eastern and central United States confirms the existence of four species: *Bufo americanus*, *B. terrestris*, *B. fowleri*, and *B. woodhousii*.

Populations of a given species are not identical in any two localities. Most of this variation is geographically haphazard, but some characters show geographical gradients (clines).

The clines for body-foot ratio and dorsal spotting are parallel in *B. fowleri* and *B. americanus*. At the boundary line where *B. fowleri* meets *B. woodhousii* the cline, however, is interrupted.

Wherever two or more species coexist in the same area some individuals with mixtures of characters of these species are present in the population.

Experimental crossing of the toad species gives rise to hybrids which resemble the intermediate specimens found in nature. The viability and fertility of the experimental hybrids seems to be normal.

There are several physiological mechanisms which prevent an unlimited exchange of genes among the species in nature. Of these perhaps the most important is difference in breeding season. At Bloomington, Indiana, there is an early-breeding unit (*B. americanus*), a late-breeding unit (*B. fowleri*), and a population which breeds at intermediate times. Individuals with mixtures of the characters of the two species occur mainly in the latter.

Difference in the mating calls of the species is probably another isolating mechanism. In at least some instances morphologically intermediate specimens have intermediate calls. Still another mechanism is found in the different ecological preferences as to breeding sites exhibited by the species.

It is possible that the exchange of genes owing to hybridization is a phenomenon of rather recent origin, perhaps connected with the changes in the environment wrought by man.

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THE BEHAVIOR OF X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN ALLIUM ROOT TIP CELLS

KARL SAX
Arnold Arboretum, Harvard University, Jamaica Plain, Massachusetts

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THERE is considerable evidence that the initial types of chromosomal aberrations induced by X-rays have differential survival rates in subsequent cell generations. The initial aberrations include translocations and inversions, which involve only rearrangement of chromosome segments, and alterations which involve losses of chromosome segments—dicentrics, rings, interstitial deletions, and terminal deletions. Although the dicentric and ring chromosomes must constitute a large proportion of the initial aberrations, only inversions and translocations were found to survive in successive cell generations in Crepis plants which had been X-rayed at the time of seed germination (GERASSIMOVA 1937) or in the progeny of X-rayed seedlings (LEWITSKY 1940). The deficient types of spontaneous chromosomal aberrations also are eliminated in later cell generations in Allium (NICHOLS 1941). On the other hand, the dicentric chromosomes do persist in the endosperm of Zea (MCCLINTOCK 1939; CLARK and COPELAND 1940), and ring chromosomes survive during the entire somatic cycle of Zea (MCCLINTOCK 1938).

The failure of dicentric and ring chromosomes to persist in the somatic development of Crepis and Allium may be explained in several ways. The deficient types of aberrations may be lethal or deleterious, or the ends of broken rings and bridges may heal so that the chromosomes divide normally. The various chromosomal aberrations were examined in successive divisions of onion root tip cells in order to determine the cause of the elimination of certain types of aberrations. This work also permitted the analysis of several other problems including the duration of the nuclear cycle in root tips, differential sensitivity, the primary and secondary effects of X-rays, and the effect of delayed germination after irradiation.

Onion bulbs and seeds were subjected to irradiation both before and after germination. Root tips were fixed in alcohol-acetic, and the Feulgen technique was used in staining. The analysis of division figures was based largely on anaphase stages, but satisfactory "metaphase" figures were obtained by treating the roots with a 0.1 percent colchicine solution for 24 hours before fixation. Aberration frequency was based on the number of chromosomal alterations per hundred cells.

CHROMOSOMAL ABERRATIONS IN ROOT TIP CELLS

Two different types of effects are produced in cells by X-ray treatments. The first is the "primary" effect (MARQUARDT 1938), which results in the

clumping and partial fusion of chromosomes and a temporary inhibition of nuclear development. At the higher X-ray doses nuclear division is suppressed for one or two days. With lower doses there is little retardation of nuclear activity, but the clumping and fusion of chromosomes is found for several hours after irradiation.

The primary effect of X-rays is evident at metaphase in less than an hour after raying. The chromosomes are more closely associated on the metaphase plate and appear to be more contracted. At anaphase the sister chromatids separate with difficulty resulting in bridges and occasional fragments. The chromatids of such a bridge may ultimately separate, but with a high frequency of bridge formation normal segregation of sister chromatids is prevented, and broken bridges and released fragments may produce deficient daughter nuclei. With a low X-ray dosage the primary effect persists for about three hours, but at higher doses the primary effect lasts considerably longer. According to MARSHAK and HUDSON (1937) the aberrations of this type produced in onion root tips are directly related to the X-ray dosage, since the dosage curve is linear.

The "secondary" effect of X-rays involves the production of the typical chromosomal aberrations. These types of aberrations are the same as those induced in Tradescantia miscrospore chromosomes (SAX 1940). When growing root tips are rayed, only chromatid aberrations are found during the first 24 hours following irradiation. Terminal deletions of one or both chromatids are the most frequent types of alterations, although exchanges do occur between the chromatids of different chromosomes. The terminal deletion of both sister chromatids followed by lateral fusion of broken ends gives rise to the single chromatid bridges which are readily detected at anaphase.

Chromosomes rayed in the resting stage usually result in only chromosome aberrations as a result of breaks before the chromosomes are effectively split into sister chromatids. Occasionally, however, irradiation of dry seeds or dormant bulbs produces a few chromatid aberrations. In the case of the seeds there is evidence that these may be of spontaneous origin. Spontaneous aberrations in dormant bulbs are rare, and the chromatid aberrations observed must be attributed either to the occurrence of the effective split in the late resting stage or to the delay in fusion of broken ends of chromosomes until prophase is initiated and the sister chromatids are differentiated. It seems more probable that breaks may remain open for many hours or perhaps indefinitely in the completely dormant resting nucleus, and that fusions occur only with the initiation of nuclear activity. Some of the fusions may be delayed until the chromsomes are effectively split, resulting in occasional chromatid aberrations.

The chromosome aberrations include dicentrics, rings, translocations, inversions, and terminal or interstitial deletions. Translocations and in-

versions can be recognized only in extreme cases in the Allium chromosomes, and the small interstitial deletions are often not easily detected. Only the dicentric and ring chromosomes have been included in the analyses. The ratio of dicentric to ring chromosomes is about 9:1. If union of broken ends were at random, the ratio should be about 30:1. The difference between observed and expected frequencies is of the same order in Tradescantia microspores (SAX 1940). The discrepancy is attributed to spatial limitations in unions of broken ends of chromosomes.

The dicentric chromosomes may separate freely, form a criss-cross bridge, or become interlocked at anaphase. About a third of the dicentrics separate freely, and of the dicentric bridges at anaphase about one-fourth are interlocked. The ring chromosomes may separate freely, form a single large ring, or become interlocked. The acentric fragments associated with these aberrations usually lie free in the cytoplasm and are not included in the daughter nuclei.

The second and subsequent nuclear divisions following irradiation also contain chromosomal aberrations. The secondary divisions can be distinguished from the primaries by the absence or disintegration of the acentric fragments. Secondary rings and dicentrics may persist owing to the free separation of these types of aberrations at the primary division or by fusions of ends of broken bridges or rings. The relative proportions of primaries and secondaries in certain experiments and the fact that some of the secondary bridges are long and interlocked suggests that fusion of broken ends may be involved, but the evidence does not exclude the possibility that all are due to freely separating primary dicentrics. The occasional occurrence of secondary chromatid bridges does seem to indicate that fusions can occur between the ends of sister chromatids following breakage of the primary bridge. Occasionally two single and apparently independent bridges are found among chromosome secondaries, suggesting that the break in a dicentric bridge is followed by fusion between sister chromatids. The occurrence of secondary chromatid bridges must mean that the breaks induced at the preceding anaphase remain open during the resting stage, or that the anaphase chromosome is more effectively split than those in the resting stage.

Although secondary dicentric and ring chromosomes are found, their relative frequency is low, and they are rarely found after several cell generations. In one series of observations the proportion of primary and secondaries was 364 to 125, while for rings the proportion of primary and secondary dicentrics was 35 to 15 in the same material. The frequencies of all classes of aberrations for an eight day period are shown in table 1. The first aberrations to appear are the fusions caused by the primary effects of X-rays; the primary chromatid aberrations are most frequent at

from 12 to 24 hours, followed by the primary chromosome aberrations. The secondary chromatid and chromosome aberrations are found on the fourth day and persist with about the same frequency during the next four days. As indicated above, the ratio of dicentric and ring chromosomes is about the same for both primary and secondary aberrations.

TABLE 1

Chromosomal aberrations induced in onion root tip cells. Roots rayed. Dose 300r.

TIME HOURS	TOTAL ANAPHASES	FUSION BRIDGES	CHROMATID PRIM.	CHROMATID SEC.	CHROMOSOME PRIM.	CHROMOSOME SEC.	FRAGMENT ONLY	TOTAL ABER. %
1.5	151	122	0	0	0	0	0	81
12	118	0	51	0	0	0	47	83
24	154	0	42	0	2	0	15	38
48	450	0	9	0	58	0	48	26
72	346	0	4	0	26	0	11	12
96	225	0	0	2	11	4	2	8
120	78	0	0	0	2	4	0	8
168	132	0	0	1	0	7	0	6
192	229	0	0	0	0	18	0	8

In another experiment dormant onion bulbs were used so that only resting cells were rayed. The bulbs were germinated, and root tips were fixed at various stages of growth. The results are shown in table 2. Only the dicentric bridges observed at anaphase were included in the analysis. The frequency of total aberrations declined as the roots elongated, and ultimately no bridges were found at anaphase in the longest roots. Other experiments confirm these results. Both onion bulbs and Tradescantia plants examined several weeks or months after irradiation have shown no bridge formation at anaphase in somatic cells.

TABLE 2

Chromosomal aberrations induced in onion root tip cells. Dormant bulbs. Dose 1200r.

ROOT LENGTH	TOTAL ANAPHASES	DICENTRIC BRIDGES PRIMARY	DICENTRIC BRIDGES SECONDARY	TOTAL ABER. %
1 cm.	377	172	0	46
2–3 cm.	427	137	5	33
6–8 cm.	242	29	23	21
10–13 cm.	72	0	8	11
20–25 cm.	87	0	0	0

The gradual elimination of dicentric bridges in successive cell generations may be attributed either to healing of broken ends of chromosomes when the bridges break at anaphase or to the elimination of cells carrying dicentric chromosomes. In the formation of dicentric and ring chromo-

somes the released acentric fragments usually are large because breaks are more frequent in the proximal end of the chromosome arm. Thus, the healing of broken ends of broken bridges of dicentrics should result in chromosomes with shortened arms. No apparent deficiencies were observed in the chromosomes after a number of cell generations, indicating that the cells carrying dicentric and ring chromosomes usually are eliminated. The failure of such cells to survive can be attributed to the large deficiencies usually associated with the formation of rings and dicentrics. Apparently these large deficiencies, even in the heterozygous condition, inhibit the growth rate so that deficient cells are unable to compete with normal cells or with cells with balanced chromosomal alterations.

Some support of the deleterious effect of deficiencies in somatic tissue is found in X-ray treatment of seedling plants. About 50 species of various ornamental plants were rayed in the seedling stages. We had hoped to produce gross chromosome deficiencies which might result in phenotypic changes of horticultural value due to unbalanced genoms. The seedlings were grown in long narrow flats so that differential dosage could be given in a single treatment. At one end of the flat the seedlings received an X-ray dosage of 5000r and at the other end 1500r. The maximum dose killed or greatly retarded the seedlings of most species. In each case seedlings were selected which were most severely affected, but still viable. Most of these grew slowly and were aberrant in many respects for several months, but at the end of the growing season the great majority appeared to be quite normal in their gross morphological characters. Presumably the more aberrant cells were eliminated and only the more normal ones or those with balanced genoms took part in the later development of the irradiated seedlings.

The survival of dicentric chromosomes and the continuation of bridge formation in the developing endosperm of Zea might be attributed to the decreased deleterious effect of deficiencies in the triploid tissue. In order to test this hypothesis, a triploid Tradescantia was irradiated and the frequencies of aberrations were determined at intervals. The frequency of primary bridges was 22 percent; at the end of a month there were still 8 percent of secondary dicentrics, but at the end of seven weeks all division figures appeared to be normal, and no aberrations could be found.

THE EFFECTS OF DIFFERENTIAL DOSAGE AND TREATMENT

It has been shown that the aberrations involving two breaks—the rings and dicentrics—vary in frequency with the square of the dosage when the time of exposure is held constant but increase as the 1.5 power of the dosage if the radiation intensity is held constant (Sax 1940). The determination of dosage curves for aberrations induced in root-tip chromo-

somes is more difficult owing to greater variability and to technical difficulties in detecting the types of aberrations produced. The data for one of the experiments are shown in table 3. The dosage was increased by increasing the time of exposure. Although the dosage curve is not so consistent as those determined for Tradescantia microspore chromosomes, it appears that the aberration frequency increases approximately as the 1.5 power of the dosage.

The exponent of 1.5 in the dosage curve equation for root tip chromosomes suggests that the time-intensity factor is operative. The time-intensity factor has been used only with onion seeds, but with rather sur-

TABLE 3

Frequency of chromosomal aberrations in onion root tips following various X-ray dosage. Roots rayed. Fixed four days after raying.

DOSE IN r-UNITS	TOTAL ANAPHASES	DICENTRICS AND RINGS	TOTAL ABER %
150	219	8	4
300	342	30	9
600	255	68	27

prising results. Two lots on onion seeds were treated; one was rayed at 200r on each of eight days over a ten day interval, and the second lot of seed was given a single dose of 1600r at the time of the last fractional dose given lot one. The frequency of spontaneous aberrations in these seeds was 3 percent. The results of the differential dosage are shown in table 4. The fractional dosage produced twice as many aberrations as the single dose, although in previous experiments fractional dosage always decreased the aberration frequency in the chromosomes of Tradescantia microspores (SAX 1940). The results obtained with the onion seed probably are due to the storage of seed after raying. GUSTAFSSON (1937) found that the aberration frequency induced in barley seeds was increased by storage before the seeds were germinated. Presumably the first exposures of the fractional dosage not only caused the directly induced aberrations but greatly accelerated the rate of spontaneous aberrations. These spontaneous aberrations increase in frequency in older seeds or in heat treated seeds, and apparently the general physiological effect of radiation produces the same response as age or heat. No such storage effect is found in the onion bulbs. Here a rest period of nine days had no effect on aberration frequency as compared with the bulbs which were germinated immediately after raying (table 4). The spontaneous aberration rate in onion bulbs is extremely low and may be neglected in determining the net effect of radiation treatment.

The data presented in table 4 also show that the onion bulbs are much more sensitive to X-rays than are the dry onion seeds. Although the seeds were given nearly three times the dosage given the bulbs, the aberration frequency was higher in the root tips developing from the bulbs. Considering the differential dosage, the bulbs were nearly ten times as sensitive as the dry seeds, as measured by aberration frequency. In another experiment where the dry seeds and growing roots were given the same X-ray dose, the aberration frequency of the dry seed was 3 percent compared with 23 percent for the rayed roots. A comparison of dry seeds and dormant bulbs

TABLE 4

The effect of storage after X-ray treatment of onion seeds (1600r) and bulbs (600r).

MATERIAL	TREATMENT	TOTAL ANAPHASES	PERCENTAGE ABERRATIONS
Seed	1 day	301	17−3=14%
	8 days	299	31−3=28%
	Control	320	3
Bulbs	March 6	216	23%
	February 26	126	21%

given equal X-ray doses showed about a 1:10 difference in aberration frequency.

The duration of the nuclear cycle in Allium root tips may be determined from the data presented in table 1. The X-ray treatment does cause a temporary cessation of nuclear activity, but after recovery from the "primary" effect the nuclear activity seems to be normal. The time lapse between successive anaphases is about three to four days as indicated by the time between the appearance of primary and secondary aberrations.

The survival of certain monosomics and other deficiencies in the heterozygous condition suggests that irradiation of gametes or zygotes would be more effective than irradiation of seedlings in producing plants with unbalanced genoms. At sub-lethal doses some of the seedling cells will not be altered, and those cells will grow faster and replace the cells with large chromosome deficiencies. A heterozygous deficiency in the zygote would not be subject to such competition and would survive in development if not cell lethal.

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SUMMARY

Irradiation of Allium root tip cells with X-rays produces a "primary" effect which results in fusion bridges. The "secondary" effect produces breaks usually followed by the union of broken ends to produce rings, dicentrics, inversions, translocations, and interstitial deletions. Terminal deletions are produced when a break is not followed by union of broken ends of sister chromatids.

The ring and dicentric chromosomes tend to persist for several cell generations but are soon eliminated, and only cells with normal or balanced genoms survive. The elimination of ring and dicentric chromosomes appears to be due to the deleterious effect of the deletions associated with these aberrations so that they are unable to compete with normal or balanced cells.

Delayed germination of irradiated onion seed increases the frequency of chromosomal aberrations due to a physiological effect similar to the effects of age or heat, but delayed germination of irradiated onion bulbs has no effect on aberration frequency.

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A SEMI-LETHAL MUTATION IN FOWL AFFECTING LENGTH OF THE UPPER BEAK AND OF THE LONG BONES

WALTER LANDAUER
Storrs Agricultural Experiment Station, Storrs, Connecticut

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IN 1938 from matings of polydactylous fowl we found newly-hatched chicks which had an abnormally short upper beak. In some of these chicks the beak gradually became normal, while others developed a cross-beak. The latter type has been referred to in an earlier publication (LANDAUER 1938, Postscript) as probably representing a new hereditary form of cross-beak in fowl.

Further studies with this material revealed that the hatched chicks with abnormal beaks are the occasional survivors of a semi-lethal mutation. A report on the morphological expression and the hereditary transmission of this mutation is given in the following pages.

MORPHOLOGY

In late stages of embryonic development and subsequent to hatching, the upper beak of normal chicks protrudes at its tip slightly over the lower beak, the outer edge of the lower mandible fitting into the inner edge of the upper mandible. In our material the upper beak was shortened. The amount of shortening varied greatly. In the least abnormal cases the upper beak was only approximately one millimeter shorter than the lower one and appeared normal in other respects, while in the most abnormal cases the upper beak was shortened by at least half its length, and its tip was rolled under. There were all intermediate stages between these two extremes (fig. 1). The beak abnormality could be distinguished as early as the ninth day of development, but not in six-day embryos.

As will be shown later, the majority of the "short upper beak" embryos died toward the end of the incubation period. A few, however, hatched. Among these abnormal newly-hatched chicks again all stages were found from a very slight to an extreme shortening of the upper beak. The beak abnormality in itself, then, appears not to be responsible for the failure of most of these chicks to hatch. The viability of the hatched "short upper beak" chicks was below normal; those with more extreme beak deformities usually died soon after hatching, presumably on account of difficulties in consuming sufficient amounts of food. In 1939, out of 23 hatched chicks with a short upper beak 18 died during the first month of life, and only three survived to maturity; in 1940, under better brooding conditions, three out of 18 died during the first month after hatching, and six survived

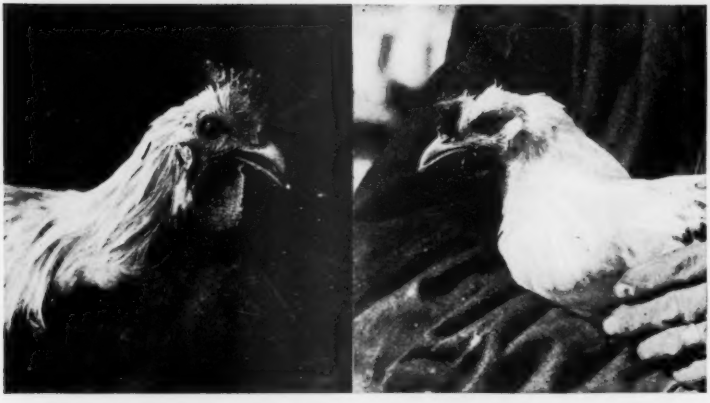

FIGURE 1 (above).—The heads of four homozygous "short upper beak" embryos, showing different degrees of shortening of the upper beak.

FIGURE 2 (left).—A mature, homozygous "short upper beak" cock (♂ 1824). The upper beak is short and also crossed toward the right side.

FIGURE 3 (right).—A mature, homozygous "short upper beak" cock (♂ 1428). The upper beak has attained normal length, but is slightly crossed toward the right side.

to maturity (table 1). Only in rare instances did adult birds or chicks which were two months of age or older show a shortening of the upper beak similar to the condition present at hatching. In nearly half of the cases the beak gradually became normal, while in most of the remaining chicks a cross-beak developed (fig. 2, 3). It is interesting to note that, as in the cases of hereditary cross-beak reported earlier, twisting of the upper beak toward the right side was much more common than twisting toward the left side. Among a total of eleven cases for which we have records, 9 showed a cross-

TABLE 1

YEAR	NUMBER OF "SHORT UPPER BEAK" CHICKS HATCHED	MORTALITY DURING FIRST MONTH	NUMBER WHICH SURVIVED TO MATURITY	CONDITION OF BEAK SUBSEQUENT TO SECOND MONTH			
				NORMAL	SHORT UPPER BEAK	CROSS-BEAK UPPER TO RIGHT	CROSS-BEAK UPPER TO LEFT
1939	23	18	3	2	1	3	0
1940	18	3	6	6	1	6	2

beak toward the right and only two a cross-beak toward the left side (table 1).

It was observed that some of the embryos and chicks with a short upper beak also had short legs. This led to a study of the length of long bones in this material. Unhatched but living normal and "short upper beak" embryos from the same matings were removed from the shells at the end of the twenty-second day of incubation. They were preserved in formaldehyde, and the long bones were dissected out and measured. The results of these measurements are shown in table 2. It may be seen that (1) the mean length of all long bones was reduced in the "short upper beak" embryos as compared with long bone length of normal embryos from the same matings; (2) in the "short upper beak" embryos the long bones of the legs were short-

TABLE 2

Average lengths of long bones in cm, together with their standard errors.

BONE	NORMAL EMBRYOS (N=23)	"SHORT UPPER BEAK" EMBRYOS (N=21)	DIFFERENCE
Femur	1.97±0.024	1.81±0.031	0.16±0.04
Tibia	2.87±0.029	2.48±0.044	0.39±0.05
Tarsometatarsus	2.13±0.028	1.70±0.032	0.43±0.04
Humerus	1.48±0.014	1.38±0.010	0.10±0.02
Ulna	1.39±0.014	1.25±0.011	0.14±0.02
Carpometacarpus	0.97±0.014*	0.86±0.012	0.11±0.02

* N=22.

ened to a greater extent than those of the wings; (3) in the leg a proximo-distal gradient existed, the most proximal bone (femur) being least reduced, while the most distal one (tarsometatarsus) showed the greatest degree of shortening.

As a check on these results, measurements were taken also of long bone length of normal and "short upper beak" embryos which had died between the eighteenth and twenty-second day of incubation. This material came from the same matings as that shown in table 2. The following average measurements in centimeters were obtained for 23 normal and 40 "short upper beak" embryos:

	NORMAL	"SHORT UPPER BEAK"
Femur	1.85±0.029	1.84±0.015
Tibia	2.61±0.045	2.50±0.021
Tarsometatarsus	1.99±0.030	1.68±0.015
Humerus	1.36±0.018	1.38±0.010
Ulna	1.28±0.014	1.25±0.010
Carpometacarpus	0.89±0.012	0.88±0.009

Since the exact age of these embryos at death was not known, no direct comparison of normal and "short upper beak" embryos was justified. However, it is interesting to compare these values with those in table 2. It may be seen that for the "short upper beak" embryos the averages of bone length of the living and dead embryos agree very closely. For the normal embryos, on the other hand, the long bones of the embryos which had died were all shorter than those of the embryos which were still alive at the end of the incubation period. This was to be expected because of the younger age of the dead embryos. The fact that no such difference existed between the two groups of "short upper beak" embryos may be taken as an indication that little or no growth in bone length occurred in these embryos toward the end of incubation.

The differential degree to which the various long bones of the "short upper beak" embryos were affected may be best illustrated by indices (table 3). The calculations are based on the same material as those in table 2. The differences between the indices for "short upper beak" and normal embryos demonstrate clearly that in the leg the tarsometatarsus was more shortened than the femur or tibia and that the tibia was more shortened than the femur. In the wings the differences were less conspicuous; yet it appears that here a gradient existed also, both ulna and carpometacarpus being shortened more drastically than the humerus. No difference in degree of shortening was found between the last two segments of the wing: ulna and carpometacarpus.

TABLE 3

Average indices of long bone length, together with their standard errors.

INDEX	NORMAL EMBRYOS	"SHORT UPPER BEAK" EMBRYOS	DIFFERENCE
$\frac{\text{Tarsometatarsus} \times 100}{\text{Femur}}$	108.0±0.54	94.2±1.23	13.8±1.34
$\frac{\text{Tibia} \times 100}{\text{Femur}}$	145.8±0.78	137.1±1.27	8.7±1.49
$\frac{\text{Tarsometatarsus} \times 100}{\text{Tibia}}$	74.2±0.36	68.8±0.93	5.4±0.99
$\frac{\text{Carpometacarpus} \times 100}{\text{Humerus}}$	65.8±0.74	62.5±0.84	3.3±1.12
$\frac{\text{Ulna} \times 100}{\text{Humerus}}$	93.9±0.61	90.6±0.80	3.3±1.01
$\frac{\text{Carpometacarpus} \times 100}{\text{Ulna}}$	70.2±0.92	69.1±1.09	1.1±1.43

It was of interest to know whether or not a correlation exists between the degree of abnormality of the upper beak and the extent to which the long bones were shortened. In order to obtain information on this question the embryos were grouped into two classes according to the more or less extreme reduction of their upper beak, and the average length of the leg bones was then calculated for each of the two groups. The differences in bone length between the two groups were very slight and not consistently in one direction. Hence, it does not appear that there is a correlation between the degree to which beak and long bones deviated from normal.

The progenies in which the "short upper beak" embryos occurred showed also segregation for polydactyly. The presence or absence of the polydactylous condition apparently had no influence on the degree to which the long bones of the legs were shortened.

Determinations of body weight were made for embryos of 15, 18, and 20 days of incubation. The averages obtained are given in table 4. These data

TABLE 4

Average body weights of embryos in grams, together with their standard errors.

AGE IN DAYS	NORMAL EMBRYOS		"SHORT UPPER BEAK" EMBRYOS		DIFFERENCE
	NUMBER	WEIGHT	NUMBER	WEIGHT	
15	37	10.67±0.20	26	10.66±0.18	0.01±0.27
18	47	20.74±0.27	14	18.54±0.44	2.20±0.52
20	144	26.85±0.24	57	25.47±0.40	1.38±0.46

indicate that toward the end of incubation there was a tendency for the average weight of the "short upper beak" embryos to fall somewhat below that of normal embryos from the same matings. This agrees well with our observations on long bone growth reported above.

TABLE 5

Results of matings of heterozygous birds inter se.

MATING	NUMBER OF FERTILE EGGS	EMBRYO MORTALITY DURING INCUBATION DAYS			HATCHED		DEAD EMBRYOS (19–22 DAYS)	
		1–6	7–18	19–22	NORMAL	"SHORT UPPER BEAK"	NORMAL	"SHORT UPPER BEAK"
19—1939								
Actual	193	11	13	52	111	6	23	29
percentage		5.7	6.7	26.9	57.5	3.1	11.9	15.0
31—1939								
Actual	174	6	6	57	94	11	15	42
percentage		3.4	3.4	32.8	54.0	6.3	8.6	24.1
33—1939								
Actual	169	16	9	48	92	4	19	29
percentage		9.5	5.3	28.4	54.4	2.4	11.2	17.2
24—1940								
Actual	206	20	9	90	86	1	46	44
percentage		9.7	4.4	43.7	41.7	0.5	22.3	21.4
All matings								
Actual	742	53	37	247	383	22	103	144
percentage		7.1	5.0	33.3	51.6	3.0	13.9	18.5
Results of matings of heterozygous ♀♀ × homozygous ♂♂								
Totals of three matings—1940								
Actual	294	19	25	136	98	16	33	103
percentage		6.5	8.5	46.3	34.1	5.4	11.2	35.0
Results of matings of normal ♀♀ × homozygous "short upper beak" ♂								
24—1940								
Actual	73	7	4	5	57	0	5	0
percentage		9.6	5.6	6.8	78.1	0	6.8	0

The conclusion that toward the end of incubation development proceeds at a lower rate than normal in the "short upper beak" embryos is further confirmed by the observation that in twenty-day embryos of this kind the yolk sac was in most cases much less completely resorbed into the body cavity than was true for normal embryos. It was also found that out

of 133 normal twenty-day embryos 111 or 83.5 percent had pierced the inner shell membrane, while out of 56 "short upper beak" embryos of the same age only ten or 17.9 percent had done so.

INHERITANCE

Female progenies of crosses in which "short upper beak" embryos had occurred were bred to males which were known to give such offspring. The results for those females which produced "short upper beak" embryos among their progenies are shown in table 5. It is unnecessary to discuss each mating separately. There was a total of 742 fertile eggs, and of these 405 or 54.6 percent hatched. Among the embryos which had died between the eighteenth and twenty-second day there were 144 or 18.5 percent with a short upper beak. In addition, there were 22 or 3.0 percent of hatched chicks with an abnormal upper beak. Of all embryos which had survived to the eighteenth day of incubation (N = 652), 166 or 25.5 percent had shortened upper beaks. From females which did not produce abnormal embryos or chicks when bred to a heterozygous "short upper beak" cock, a total of 675 fertile eggs were obtained. Embryo mortality during the three incubation periods amounted to 5.0, 4.6, and 14.5 percent, respectively, and there were 75.9 percent hatched chicks. These segregation data agree well with the assumption that the shortening of the upper beak (and the other abnormalities associated with this trait) are caused by a recessive mutation which has a semi-lethal effect.

During 1940 it was possible to make crosses of females which were known to be heterozygous for the "short upper beak" mutation and males which had had a short upper beak at hatching time and hence, presumably, were homozygous for the trait. The results of these crosses are given in table 5. Two hundred ninety-four fertile eggs were obtained, of which 114 or 39.5 percent hatched. Among the embryos which died between the eighteenth and twenty-second day there were 103 or 35.0 percent with a short upper beak; 16 or 5.4 percent of such specimens hatched. Out of a total of 250 embryos which survived to the end of the eighteenth day 119 or 47.6 percent had a short upper beak. This result agrees sufficiently well with the expected 50 percent of abnormal embryos and chicks to serve as verification of the assumption that the "short upper beak" trait is due to a single recessive gene difference.

A homozygous "short upper beak" cock was mated to hens which were known not to carry the mutation (table 5). Seventy-three fertile eggs were obtained, and 57 of these or 78.1 percent hatched. No abnormal embryos or chicks were produced. Hence, we are dealing with an autosomal mutation.

From the data in table 5 it appears that there was little, if any, prefer-

ential mortality of "short upper beak" embryos up to the end of the eighteenth day of incubation. Further observations indicate that about one third of the "short upper beak" embryos which survived the eighteenth day were still alive when the unhatched eggs were opened at the end of the twenty-second day, while most of the remaining embryos apparently died on the twenty-first and twenty-second day. The hatchability of those "short upper beak" embryos which survived the eighteenth day amounted to 13.3 percent (22 out of 166) in the matings of heterozygous parents and to 13.4 percent (16 out of 119) in the matings of heterozygous females by homozygous males.

In the three matings of heterozygous females by homozygous males fertility of the eggs was low. The actual data are as follows.

PEN	TOTAL EGGS SET	NUMBER OF INFERTILE EGGS	FERTILITY PERCENTAGE
1–1940	58	36	37.9
12–1940	618	241	61.0
25–1940	474	405	14.6

At the end of the regular hatching season the cocks in pens 1 and 25 were used for artificial insemination. Although both animals readily responded to stimulation, only small quantities of sperm were obtained. It would be interesting to know whether this low fertility is due to the beak abnormality (all three males had cross-beaks) and a consequent deficiency in food consumption or whether it is more directly a physiological concomitant of the "short upper beak" mutation. No effect on fertility was observed in our earlier breeding experiments with hereditary cross-beak of fowl. In two matings of normal females by cross-beak males[1] the fertility of eggs amounted to 62.5 percent (477 eggs set) and to 76.0 percent (367 eggs set), respectively. More critical information, however, is needed to settle this problem.

DISCUSSION

A number of semi-lethal mutations exist in which occasional survivors reach the adult stage; in most instances such individuals are sterile. In fact, except for Drosophila, no matings seem to have been reported involving homozygous semi-lethal animals. In Drosophila, at least two sex-linked semi-lethal mutations are known (MORGAN 1929; BONNIER and NORDENSKIÖLD 1936) in which homozygous semi-lethals of one or both sexes have been bred. The only autosomal semi-lethal of Drosophila for which fertility has been reported is "curly" wing (WARD 1923), though in this case no segregation ratios are on record.

[1] Both males had the cross-beak which appears after hatching (type III; LANDAUER 1938).

The stock in which the "short upper beak" mutation was found traces back to Houdan ancestry, but they were not pure Houdans. DR. R. J. BUSHNELL kindly informed the writer that in pure Houdans he has observed embryos with the same kind of beak abnormality. It is likely, then, that the mutation with which we worked was derived from Houdan fowl. This is of interest because, as in the Cornish breed, Houdans are required by the standard of breeders to have rather short legs. Though we have no metric data as yet, there is some evidence suggesting that the effect which the "short upper beak" mutation has on long bone length may have a slight degree of expression in the heterozygous condition. If this is verified, we shall have another instance in which, as in Creeper and Cornish fowl, a reduction in length of the legs is maintained, at least in part, at the expense of propagating a lethal mutation.

The morphological features of the "short upper beak" mutation are of great interest in relation to the other lethal mutations of fowl. There are at present seven chicken lethals on record for which the morphological expression is known. No less than five of these seven mutations affect simultaneously head and extremities. These are (1) the early and late stages of the homozygous Creeper mutation (LANDAUER and DUNN 1930; LANDAUER 1933); (2) the Cornish lethal (LANDAUER 1935); (3) the lethal producing extreme polydactyly (COLE 1939); (4) a new lethal which according to a brief communication by ASMUNDSON (1939) causes a deformity of the lower beak, curving of the upper beak, and shortening of the legs; and (5) the semi-lethal mutation described in the present report. After X-ray treatment of sperm (testes), KRAYEVOY (1938) frequently found F_1 embryos in which abnormalities of head and extremities existed together.

A similar situation is met with among non-hereditary variations. Thus, association of head and appendicular abnormalities is shown by sporadic chondrodystrophy of chicken embryos (LANDAUER 1927; DUNN 1927); by embryos and chicks produced on manganese-deficient diets (BYERLY, TITUS, ELLIS, and LANDAUER 1935; LANDAUER 1936; CASKEY and NORRIS 1940); and by embryos developing in riboflavin-deficient eggs (ROMANOFF 1940). The simultaneous occurrence of malformations of head and extremities is also the most common type of embryonic abnormality produced by the ingestion of selenium by laying hens (LANDAUER 1939, c). In mammals, including man, considerable evidence is also on record for the occurrence of similar syndromes, involving head and extremities, of hereditary and of non-genetic nature.

A shortening of the upper beak occurs commonly as a non-hereditary abnormality (phenocopy) under the influence of unfavorable incubation conditions, but it is not known whether or not in this case the shortening of the upper beak is associated with a shortening of the long bones.

The occurrence of abnormalities of this type is not limited to fowl ASMUNDSON (1939), for instance, found a recessive mutation among Bourbon turkeys which brings about a reduction in length of the lower beak and of the legs. Among pigeons, the first two lethals were discovered recently. One of these mutations produces polydactyly and shortening of the upper beak (semi-lethal), and the other gives rise to micromelia (chondrodystrophy ?) and abnormal conformation of the head.[2] Again, it is scarcely coincidence that the first two lethal mutations to be found in this genus should both show associated abnormalities of head and extremities. Interesting in this connection also are observations made by DARWIN (1876) on tumbler pigeons. He found in a comparison of different breeds of tumbler pigeons that a correlation exists between length of the (whole !) beak and length of the foot. Unfortunately, nothing is known concerning the developmental or genetic aspects of this correlation.

It cannot be assumed that all these different hereditary and non-genetic agencies interfere in a specific and identical manner with developmental processes involved in the formation of head and extremities. Alternatively, it seems reasonable to conclude that these embryonic parts are especially susceptible to disturbances of the most varied nature, and this high susceptibility may have its basis in the intense growth activity of these parts during early developmental stages. Toward the end of development, growth of the whole body also falls below normal.

Another point of interest relates to the differential effect on length of different long bones. Measurements are available for three of the chicken lethals which affect long bone length (Creeper, Cornish, "short upper beak"), and in all three it was found that (1) the leg bones are more involved than the wing bones, and that (2) a proximo-distal gradient exists within the legs, the femur being least reduced and the tarsometatarsus most. A similar situation is encountered in the non-hereditary micromelia produced in embryos and chicks by manganese deficiency of the eggs. These similarities of morphological response to three independent mutations and to a nutritional deficiency must have their causes in some fundamental developmental pattern of the chicken embryo.

Such mechanisms, however, may operate also subsequent to hatching. In dwarfism of fowl produced by hereditary thyroid abnormality (LANDAUER 1929; UPP 1934) the same type of gradient of shortening of the leg bones is found.

It has been shown by LERNER (1936, 1937) that a growth gradient exists in the leg of fowl, "with the distal bone (tarsometatarsus) growing at a more rapid rate in relation to the rate of body growth, the median bone (tibiotarsus) presenting an intermediate rate of growth, and the proximal

[2] Personal communication from DR. W. F. HOLLANDER, Sumter, S. C.

bone (femur) the slowest." These relations, of course, imply the existence of corresponding differences in growth intensity of the three bones, irrespective of body weight. It has also been shown by LERNER that in these bones essentially the same growth relations hold for the embryonic and post-hatching period. There can be little doubt that this normal growth gradient and the differential response of the leg bones to the shortening effect of the hereditary and non-genetic agencies under discussion have a common material basis.

Even more illuminating, especially with reference to our observations on "short upper beak" embryos, are data on the relative growth of nestling house wrens recorded by HUGGINS (1940). Some of the growth constants (α) which she found are as follows: bill 1.3205, body 1.0167, tail 1.0551, femur 1.2863, tibiotarsus 1.4106, tarsometatarsus 1.5778,humerus 1.3270, radius-ulna 1.4914, manus 1.2150. The beak and the long bones show much higher growth constants than the body proper. These are the parts which are affected by the "short upper beak" mutation of fowl. The long bones of the leg in the wrens, as in chickens, show a proximo-distal gradient of increasing growth intensity. It has been pointed out already that a similar gradient exists with reference to the amount of reduction in length of the long bones under the influence of the mutations in question. No clear-cut proximo-distal gradient was found in the wing of the house wren, and this again agrees with the less clear-cut differential effect of the "short upper beak" mutation on the wing bones. HUGGINS's data on relative growth of wren nestlings, however, do not furnish a basis for explaining the much lesser effect of the "short upper beak," Cornish, and Creeper mutations on wing bones as compared with leg bones. This apparent discrepancy is probably due to the fact that at the beginning of the post-hatching period the growth rate of the wing bones becomes much greater, relative to the leg bones, than it is during the embryonic period (STREICH and SWETOSAROW 1937), and it is, of course, growth during embryonic development which interests us with regard to the effect of the lethal mutations and of other factors interfering with normal development.

The foregoing evidence indicates clearly that the responses of parts of the embryo to the several mutations are influenced, at least in certain respects, by the inherent normal growth characteristics of these parts. Do such differential responses lead only to size differences or are they responsible also for structural peculiarities?

Evidence obtained with Creeper fowl has some bearing on this question and may usefully be discussed here. The writer in previous publications proposed that the various abnormalities of organs and parts which are found in homozygous Creeper embryos might be secondary effects of an arrest in growth of the whole embryo. More recently (LANDAUER 1941), the interpretation that the fate of parts is determined by alterations in the

embryo as a whole has been abandoned. It has been pointed out instead that "the localized effects are due merely to quantitative differences in physiological needs of certain parts at definite periods; these differences depending on such factors as differential growth activity, involving differential needs for certain enzymes and other substances."

In a discussion of his transplantation results with limb primordia of normal, heterozygous, and homozygous Creeper embryos, HAMBURGER (1941) admits the universal nature of the action of the Creeper mutation but thinks that its effect is probably due to "some deficiency in a general metabolic or respiratory mechanism" which "might concern a specific reaction or substance involved in such a general activity." It seems to the present writer that the two interpretations are in virtual agreement. HAMBURGER takes exception to the writer's continuing emphasis on growth processes as probably being an especially important agency in calling forth differential responses. However, his failure to obtain Creeper phenocopies by the transplantation of normal limb buds cannot be taken as proof against such an interpretation. Growth is the result of the complex interaction of many different processes. Interference with any of a great number of physiological reactions contributing to growth may result in disproportionate reduction of the size of certain parts but may at the same time, according to differential needs of these parts, result in specific structural alterations in each instance.

The experiments of FELL and LANDAUER (1935) have shown that many of the peculiarities of late homozygous Creeper extremities may be reproduced in genetically normal limb buds by exposing them to an abnormal culture medium. Similarly, DORRIS (1938 and personal communication) observed that eyes of genetically normal embryos when cultured in vitro tend to form retinal "rosettes," and that (in plasma clot cultures, but not in liquid media) there is frequent failure in the formation of the scleral cartilage—two abnormalities which are frequently found in late homozygous Creeper embryos. Furthermore, the structural asymmetries of eyes and otocysts on the left and right side of early homozygous Creeper embryos (RUDNICK and HAMBURGER 1939) are likely to have their origin in asymmetries of growth activity which are known to exist on the two sides of the head region (OLSEN and BYERLY 1935) and which may produce their effect by the creation of differential needs for specific substances. Evidence from other sources also supports the conclusion that morphogenetic and histogenetic processes may be modified by alterations in general conditions of body growth (SCHMALHAUSEN 1925; IRICHIMOWITSCH 1936; and others).

From evidence now available it seems likely that the "short upper beak" mutation belongs to the same category as the Creeper mutation in so far as the fundamental effect of the genic alteration is probably one which affects a developmental process common to the whole embryo.

SUMMARY

A new lethal mutation in fowl is reported which produces a shortening of the upper beak and of the long bones of the extremities.

The mutation is recessive, autosomal, and semi-lethal. The majority of homozygous embryos die near the end of the incubation period, but about 13 percent of all homozygotes hatch.

The degree of shortening of the upper beak and of the long bones is variable, and there is apparently no correlation between the extent to which the two structures are affected.

In many of the hatched "short upper beak" chicks the beak becomes normal during growth. In others, a cross-beak develops, the curvature of the upper beak being more frequently toward the right than toward the left side. In a few, the shortness of the upper beak persists to maturity.

Among the hatched homozygotes there is a high mortality, presumably on account of difficulties in eating. Adult "short upper beak" cocks give low fertility.

The shortening of the long bones affects the wing bones less than those of the legs. Within the leg the shortening of the individual bones follows a proximo-distal gradient, the femur being least affected and the tarso-metatarsus most.

The shortening of the upper beak has been seen in embryos as early as nine days, but not in six-day embryos.

In late stages of development body weight of the homozygotes is below that of normal embryos. There are other signs that the rate of development falls below normal toward the end of incubation (delayed resorption of the yolk sac, frequent failure of embryos to pierce the inner shell membrane). These late manifestations of the "short upper beak" mutation presumably are another expression of the fundamental disturbances which occur early in development.

The developmental aspects of the "short upper beak" mutation are discussed, with special reference to similarities with the Creeper mutation. It is concluded that in both instances the mutations are responsible for aberrations in physiological mechanisms which are common to the whole embryo.

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STUDIES ON MODIFIER OF BAR. A MUTANT WHICH AFFECTS THE EXPRESSION OF THE BAR MUTATION OF DROSOPHILA MELANOGASTER

ARTHUR G. STEINBERG[1]
Columbia University, New York

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INTRODUCTION

TEMPERATURE experiments on Bar (a semi-dominant sex-linked mutant in *Drosophila melanogaster*) have shown that the facet number of Bar varies inversely with temperature and that temperature is effective during only a limited period of the larval stage. A facet inhibiting reaction with a Q_{10} higher than that of the rest of the developmental processes was postulated to explain these data (see GOLDSCHMIDT 1938 and BEADLE 1939 for review and literature). The temperature effective period was considered to be the period during which the reduction in facet number by virtue of the facet inhibiting reaction occurred.

By a study of the growth curves of the eye discs of Bar, modified Bar (see below) and wild type and of the effect of different temperatures on the size of the eye discs, it has been found (STEINBERG 1941) that the temperature effective period is not primarily responsible for the reduction of facet number in Bar. On the basis of the data there presented, it was postulated that the range of facet number over which an eye is able to vary is determined at the time of origin of the cephalic complex (that is, in the egg stage) and that the exact facet number is not determined until pupation. It was pointed out that several extrinsic factors, temperature being one, are capable of affecting the final facet number in Bar. It was postulated that these factors are able to do this because a group of cells is labilely determined to form either facets or head chitin and that the factors affecting facet number simply shift the path of development of this labilely determined tissue.

The mutant to be reported in this paper (modifier of Bar, $m(B)$) has an effect on facet number only in the presence of some one of the Bar mutants. Inasmuch as the modifier was found to have no effect on either optic disc size or cell size, it was assumed that it has no effect on the number of cells which are labilely determined—that is, the number of labilely determined cells in Bar and modified Bar are assumed to be the same. The modifier is considered to be an intrinsic factor which affects the path of development of the labilely determined cells in such a way that more of them form facets than do so in its absence.

[1] Now at Department of Genetics, McGill University, Montreal.

Although the evidence presented in the previous paper (STEINBERG, 1941) strongly indicated that the modifier affected facet number by shifting the path of development of labilely determined cells, it by no means proved this, and experiments testing this point are therefore desirable.

This paper is a presentation of data concerning the mode of inheritance of *m(B)* and the nature of its interaction with various mutants affecting eye size.

Modifier of Bar (STEINBERG 1937a, b; STEINBERG and ABRAMOWITZ 1938) was found during the course of some unpublished experiments in which Bar was used as a marker and crossing over was measured in the "all" complex of the second chromosome. The cross was XXY; "all" /+ ♀ ♀ by *B;* "all"/*dp*²*Cy* ♂♂ ("all" = *al* (aristaless), *dp* (dumpy), *b* (black), *pr* (purple), *c* (curved), *px* (plexus), and *sp* (speck); *Cy* = Curly).

Crossing over was followed in the non-*Cy* "exceptional" offspring. Those males showing Bar were of course "exceptional," having their X chromosome from their fathers. The experiment was discontinued shortly after its inception because SCHULTZ (MORGAN, BRIDGES, and SCHULTZ 1935) at that time reported the results of a similar type of experiment and the data of the present author were simply confirming his. In three different cultures exceptional males with large Bar eyes appeared.

Culture No. 211 yielded only one exceptional male. It was phenotypically *B; px sp*. It had exceptionally large Bar eyes.

Culture No. 215 yielded three exceptional males. One was *B;++* and had the usual type of Bar eyes; another was *B; px sp*, its eyes were similar to those of the male from culture No. 211; the third was *B; al dp b px sp* and it also had large eyes.

Culture No. 232 yielded one exceptional male; it was *B; px sp* and had large eyes.

Other exceptional males (with "normal" Bar eyes) were as follows:

Culture No.	Phenotype
205	*B;+*
212	*B; al dp b pr*
214	*B; sp*
220	*B;+*
224	*B; al dp b*
	B; c px sp
227	*B; px sp*
228	*B; al*
230	*B; b*

From the nature of the cross, all the males must have had the "all" complex in one second chromosome. Only those males homozygous for

px and *sp* showed the large eye (see cultures 211, 215, 232); however, the presence of *px* and *sp* did not necessarily lead to a large eye, as evidenced by cultures No. 224 and 227.

It seems likely from these data that the "all" chromosome in some cases carried a modifier of Bar which is close to *px* and *sp*. Direct tests for the location of *m*(*B*) reported below confirm this.

The *B; px sp* males from cultures 211 and 215 were mated to Oregon-R (Or-R) wild type females. In the F_1, heterozygous Bar females were mated to sib males. From this mating *B; px sp* males and *B*/+; *px sp* females were selected and a stock started.

LINKAGE GROUP

To determine the linkage group to which *m*(*B*) belongs females homozygous for Bar, modifier of Bar, plexus and speck were crossed with *Cy*/*Pm*; *H*/In(3R)Mo, *Sb sr* males (*Cy* = Curly wings, a mutant associated with inversions in both arms of chromosome II; *H* = Hairless, In(3R)Mo = inversion in 3R, *Sb* = stubble bristle, *sr* = stripe). F_1 *B; Cy; Sb* males were backcrossed to females like their mothers. To avoid facet counts, an arbitrary grading system was adopted in which the largest modified Bar eye was graded as 4 and "normal" Bar eye as 1.

Two such experiments were performed. In the first the F_1 flies were not graded for eye size. However, their eyes were observed to be small (like "normal" Bar). In the second experiment the F_1 males were classified as to eye size, *Cy*, *Pm*, *H*, and *Sb*. In both experiments the backcross male offspring were classified as to eye size, *Cy* and *Sb*. All males were, of course, genotypically *B*. Table 1 shows the results of these crosses, all of which were made at 25 ± 1°C. The eyes of F_1 males of all four classes appear to be somewhat larger than the "normal" Bar, having an average eye size rang-

TABLE 1

Summary of the data for the location of the linkage group to which m(B) belongs. See text for further explanation.

Cross: *B/B; m(B) px sp/m(B) px sp* ♀ × +; *Cy/Pm; H/C Mo Sb* ♂

F_1 ♂ phenotype	*B*				N
	Cy; H	*Pm; Sb*	*Cy; Sb*	*Pm; H*	
Average grade (Exp. 2)	1.0	1.5	2.0	1.4	80

Cross: F_1 *B; m(B) px sp/Cy;* +/*C Mo Sb* ♂ × *B/B; m(B) px sp/m(B) px sp* ♀

Phenotype	*B*				N
	Cy; +	+; *Sb*	*Cy; Sb*	+; +	
Average grade (Exp. 1)	1.5	4.0	1.9	3.7	34
(Exp. 2)	1.9	3.9	1.9	3.7	592

ing from 1.4 for *Pm; H* to 2.0 for *Cy; Sb*. There can be no doubt, however, that they are smaller than modified Bar. The modifier therefore is not dominant, although it may be semidominant. The backcross data for both experiments clearly show that the modifier is located in the second chromosome, since in the absence of *Cy* (that is, when both second chromosomes are derived from the modified Bar stock) the average eye size ranges from 3.7 to 4.0, in good agreement with the eye size of the modified Bar stock. In the presence of *Cy*, however, the average eye size varies from 1.5 to 1.9, well within the range of the F_1.

Since both plexus and speck are in the second chromosome and are present in the modified Bar stock, the question arises as to whether either or both of these are the cause of the increased facet number shown by modified Bar. To test this possibility, modified Bar, plexus, speck females (*B/B; m(B) px sp/m(B) px sp*) were crossed to Curly/plexus, brown, morula, speck males (*Cy/px bw mr sp*). The F_1 males were all *B* and either *Cy* or *px sp* (phenotypically). Twenty-five of each type were graded with regard to eye size. The *Cy* males had an average grade of 1.5; the *px sp* males 1.7. These values compare favorably with those found for the F_1 males and the *Cy* males of the backcross reported in table 1. Therefore *px* and *sp* are not the cause of the increased facet number, and hence their presence may be ignored in the experiments to be reported below.

Unpublished data of MR. CHARLES HENDLEY indicate that *m(B)* is located between 6 and 7 units to the left of *px*.

FACET COUNTS OF BAR AND MODIFIED BAR AT 25° AND 29°C

The Bar stock used in the following experiments had been rendered isogenic with an inbred Florida wild type stock and then maintained for more than 100 generations by brother sister matings before these experiments were begun. This stock was the source of the Bar mutant in the *B/B; m(B) px sp/m(B) px sp* stock. The latter stock had been inbred for about ten generations before the facet counts were made. No attempt was made to render it isogenic with the Bar stock. Facet counts were made of Bar and modified Bar at 25 ± 1°C and 29.2 ± 0.1°C. Eggs were collected from six to ten females in large shell vials over a 24 hour period at 25 ± 1°C. The females were then removed and the vials transferred to the desired temperature, where they were left for the remainder of the experiment. The food consisted of the standard cornmeal-agar-molasses medium to which 1.5 percent of dried brewers yeast had been added. Facet counts were made by dissecting off the cornea of the eye, mounting it on a slide in a drop of water, and then projecting the image. The data are presented in tables 2, 3, and 4.

The facet counts confirm the earlier observations that the modifier has

TABLE 2

Facet numbers of Bar and modified Bar males and females at 25±1°C.

GENOTYPE	MEAN ± σ_M	RANGE	V ± σ_V	N
		Males		
B	74.2±2.7	41–129	25.1±2.78	46
B; m(B) px sp	220.9±4.8	143–315	15.2±1.55	50
		Females		
B	75.2±2.5	44–104	23.1±2.43	50
B; m(B) px sp	141.1±2.3	115–211	11.7±1.19	50

TABLE 3

Facet numbers of Bar and modified Bar males and females at 29.2±0.1°C.

GENOTYPE	MEAN ± σ_M	RANGE	V ± σ_V	N
		Males		
B	38.0±0.8	33–50	10.0±1.43	25
B; m(B) px sp	169.7±4.6	117–234	19.2±1.99	50
		Females		
B	35.7±0.6	30–44	9.5±1.15	34
B; m(B) px sp	114.5±2.4	82–174	14.6±1.49	50

TABLE 4

Facet numbers of Bar males and females heterozygous for the modifier. Temperature = 29.2±0.1°C.

GENOTYPE	MEAN ± σ_M	RANGE	V ± σ_V	N
		Males		
B; m(B) px sp/+	75.0±0.8	62–87	7.9±0.79	50
		Females		
B/B; m(B) px sp/+	56.7±0.8	42–68	9.5±0.95	50

a marked effect on Bar males and a much slighter effect on Bar females. In both sexes, however, the increase over "normal" Bar is considerable and significant. Furthermore, there is no overlap at either 25 or 29°C (tables 2, 3).

At neither temperature is there a certainly significant difference between the facet number of "normal" Bar males and females. (D/σ_D at 25°C = 0.27; D/σ_D at 29°C = 2.3.)

The situation, however, is quite different in the case of the modified Bar males and females. At 25°C modified Bar males have 79.8 more facets

than the females ($D/\sigma_D = 15.1$), while at 29°C the modified Bar males have 55.2 more facets than the females ($D/\sigma_D = 10.8$).

Comparison of the facet number of the Bar males at 25° and 29°C and of the Bar females at the same two temperatures shows that at the higher temperature as compared with the lower there has been a reduction of approximately 50 percent in facet number in both sexes. This is in agreement with the observations of earlier workers. However, when a similar comparison is made for the modified bar males and females, it is found that the reduction in the case of the former is 23 percent and in that of the latter 19 percent. These values, while not significantly different from each other ($D/\sigma_D = 1.16$), are each different from those for Bar (D/σ_D for males $= 6+$ and for female $= 9+$). Thus, while in both Bar and modified Bar an increase in temperature leads to a decrease in facet number, a change in temperature from 25° to 29° has only about half as great an effect on modified Bar as on Bar.

The facet counts on Bar males and females heterozygous for the modifier establish that the modifier is a semi-dominant (table 4). Its effect in the heterozygous state is slightly less than one third of that in the homozygous condition (at 29°C); here again the effect is greater in the males. It is worth noting that the degree of dominance is the same in both sexes; 28.1 percent in the males and 26.6 percent in the females ($D/\sigma_D = 0.8$). (The percentage dominance was determined by dividing the increase of facet number caused by the modifier in the heterozygous state by that caused by it when homozygous and multiplying by 100.)

THE EFFECT OF THE MODIFIER ON THE BAR "ALLELES," EYELESS^2 AND WILD TYPE

The effect of *m*(*B*) on the Bar "alleles" (double Bar (*BB*), infra-Bar (B^i), and double infra-Bar (B^iB^i)), eyeless^2 (ey^2), and wild-type was determined from facet counts in stocks rendered homozygous for the modifier and the mutant in question (*px* and *sp* were also present, but in view of the data presented above, they may be ignored).

These counts were made somewhat later than those on Bar, and the method of egg collection was modified. In these experiments 40 newly hatched larvae were placed in a $1'' \times 4''$ vial where they completed their development. The food was the same as in the Bar experiment. None of the stocks was isogenic. The temperature for all counts was 25 ± 1°C. In all but one case the stocks with and without *m*(*B*) were reared simultaneously. The sole exception was the second run of the $fB^iB^im(B)$ for which no special controls were counted. The data are presented in table 5.

With regard to the presence or absence of an effect of the modifier on facet number in *BB*; B^i; B^iB^i, and ey^2, the data are clear. The modifier

increases the mean facet number expressed by each of the alleles of Bar and has no effect on the mean of eyeless[2]. In the case of wild type—that is, absence of any mutant other than $m(B)$ which affects facet number—there is an effect when the Florida (+fla) stock is used as control and none at all when Oregon-R is used. (Both these wild type stocks were inbred.)

TABLE 5

Facet numbers in the B "alleles," ey² and + with and without m(B). Temperature = 25 ± 1°C.

GENOTYPE	♀♀				♂♂			
	$M \pm \sigma_M$	$V \pm \sigma_V$	RANGE	N	$M \pm \sigma_M$	$V \pm \sigma_V$	RANGE	N
BB	27.2± 0.70	15.8± 1.86	18– 34	37	28.9± 0.8	15.6± 1.97	17– 36	33
BB; m(B)	40.5± 1.05	16.0± 1.86	33– 62	38	46.8± 0.9	11.9± 1.40	37– 62	37
fB^iB^i	65.4± 1.5	16.4± 1.64	48– 99	53	66.8± 2.3	14.8± 1.65	50– 98	42
(a) fB^iB^i; *m(B)*	166.1± 9.0	34.3± 4.26	81–344	40	140.6± 6.2	29.1± 3.30	80–263	43
(b) fB^iB^i; *m(B)*	143.4± 5.9	29.5± 3.13	80–283	52	131.4± 3.7	21.0± 2.05	84–204	57
Average of a and b	153.3± 5.1	32.2± 2.61	80–344	92	135.4± 3.4	25.1± 1.88	80–263	100
fB^i	342.1± 14.5	21.6± 3.13	223–530	26	345.3± 8.6	12.7± 1.79	226–460	26
fB^i; *m(B)*	679.6± 9.1	9.3± 0.95	529–850	48	691.4± 7.3	6.7± 0.74	558–821	41
+Fla	746.4± 3.6	2.5± 0.34	717–798	27	680.6± 2.7	2.0± 0.28	658–707	25
+Or-R	833.4± 5.0	3.0± 0.42	767–872	25	765.2± 4.2	2.7± 0.38	703–790	25
+; *m(B)*	826.0± 4.6	3.2± 0.39	729–882	34	766.1± 3.1	2.3± 0.29	734–801	31
ey^2	377.4± 12.3	20.8± 2.46	199–519	41	363.6± 10.7	19.7± 2.16	186–486	45
m(B); ey^2	347.3± 22.5	39.5± 5.33	146–619	36	380.8± 23.0	38.0± 4.82	103–616	40

In view of the fact that the original modified Bar males were crossed to Or-R females and in view of the fact that the +; *m(B) px sp* stock was derived by crosses with Or-R, it seems to the author that the comparison with Or-R is the more valid one, and therefore it is concluded that $m(B)$ has no effect on mean facet number in the absence of some one of the Bar "alleles."

DISCUSSION

So far as the present author is aware, there is only one other major modifier of Bar known, namely minusbar reported by NORDENSKIÖLD (1934). It, however, is located in the third chromosome and appears to have a similar effect on males and females and therefore cannot be the same as the one reported in this paper.

Modifier of Bar ($m(B)$) is probably a point mutation for the following reasons: (1) It has little or no effect on viability or fertility when homozygous and is viable when opposite a deficiency for its locus (unpublished data of P. N. BRIDGES); therefore it is probably not a deficiency. (2) Although it is close to *px*, it has no effect on recombination between *px* and *sp;* thus it is not likely to be an inversion. (3) It does not affect random

assortment between the X, II and III chromosomes (see table 1); therefore, if it were a translocation, it could be only a II-IV translocation. This, however, is unlikely because of its viability and fertility when homozygous and because of (2) above. The possibility of its being a short duplication such as Hairy wing or Bar is of course not eliminated.

EFFECT OF $m(B)$ ON FACET NUMBER IN BAR AND DOUBLE BAR

There is at present no satisfactory explanation of the vastly greater effect of the modifier in Bar males than in Bar females (tables 2, 3, 5, 6). It may be pointed out, however, that a nitrogenous extract of Calliphora

TABLE 6

Ratios of facet numbers of the Bar "alleles" with and without the modifier. Temperature = 25±1°C.

GENOTYPE	♀♀ RATIO $\pm\sigma_R$	♂♂ RATIO $\pm\sigma_R$
$\frac{B;\, m(B)}{B;\, +}$	1.88±0.069	2.98±0.125
$\frac{BB;\, m(B)}{BB;\, +}$	1.49±0.055	1.62±0.055
$\frac{fB^iB^i;\, m(B)}{fB^iB^i;\, +}$	2.34±0.093	2.03±0.085
$\frac{fB^i;m(B)}{fB^i;\, +}$	1.99±0.087	2.00±0.054

larvae which when fed to Bar larvae causes an increase in facet number also has a greater effect on males than on females (EPHRUSSI, KHOUVINE, and CHEVAIS 1938; CHEVAIS and STEINBERG 1938) as does also pure oxygen atmosphere (MARGOILIS 1939).

The data of tables 2 and 3 indicate that temperature has much less effect on the facet number of modified Bar than on that of "normal" Bar. If, as postulated above, facet number within a given genotype is shifted by changing the path of development of a group of labilely determined cells, both the modifier and temperature are acting on the same cell-group and in opposite directions. It is to be expected therefore that some compromise would be reached—that is, an intermediate number of facets would result.

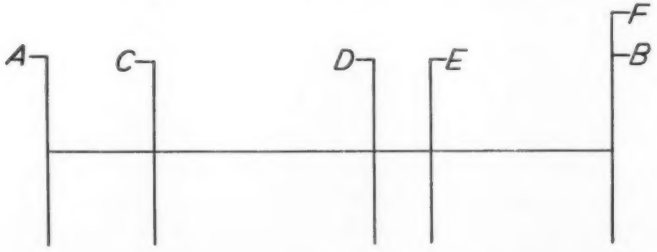
With regard to double-Bar it seems necessary merely to indicate that the effect of the modifier is not so great on it as on Bar (tables 2, 5, 6) and that there does not appear to be any difference between the effect of the modifier on the two sexes (table 6). (Facet number counts were made at only one temperature. But it has been shown by other workers that double Bar responds to temperature in the same manner that Bar does, and therefore

it may be predicted that the response of modified double Bar to temperature will be similar to that of Bar—that is, an intermediate number of facets.)

EFFECT OF $m(B)$ ON FACET NUMBER IN INFRA-BAR AND DOUBLE INFRA-BAR

The data of table 5 show that in the presence of $m(B)$ the facet number of infra-Bar flies is increased (to the same extent in both sexes, compare table 6) and that the coefficient of variation is significantly reduced.

If the postulate that the changes in facet number expressed by a given mutant are the result of shifts in the path of development of labilely determined cells is correct it follows that the range of variation of which the phenotype is capable is fixed by the number of such labilely determined cells present in the eye disc. Naturally there must be both a lower and an upper limit to the potential range of variation. At 25°C in the absence of the modifier a portion of the potential range of the infra-Bar eye is realized (226 to 460, compare table 5). In the presence of the modifier another portion of the potential range is realized (558 to 821, table 5). The decreased variability exhibited by infra-Bar in the presence of the modifier may be explained by assuming that the range of variation is shifted so as to include the maximum facet number of which the eye is capable and that therefore the upper portion of its range is cut off. This may be illustrated by the following diagram. The limits of the line AB represent the complete range



of facet number which a given genotype may express (in the present discussion infra-Bar). The interval CD represents the range realized at 25°C in the absence of the modifier (compare B^i, table 5). The interval EF (F coincides with or is very close to B) represents the range realized at 25°C in the presence of the modifier (compare B^i; $m(B)$, table 5).

Facet number in both infra-Bar and double infra-Bar increases with an increase in temperature. Hence unlike the situation in Bar and double-Bar, an increase in temperature acts in the same direction as does the modifier. No facet counts were made at temperatures other than 25°C, but it is

possible to predict from the data at hand what would be the combined effect of increased temperature and the modifier. Consider infra-Bar first. (Since the situation is the same in both sexes, only the males will be discussed.) In the absence of the modifier the mean facet number was 345.3 the coefficient of variation was 12.7 ± 1.79, and the range of facet number extended from 226 to 460; in the presence of the modifier the mean facet number was increased to 691.4, the coefficient of variation was reduced to 6.7 ± 0.74, and the range changed to 558–821 (see table 5). The reduction in the coefficient of variation is significant ($D = 6.0 \pm 1.9$). (The modifier does not characteristically tend to lower the coefficient of variation but has different effects in different cases (see table 5); this will be discussed more extensively below.) Obviously a facet number greater than that at B (see diagram) cannot be realized in the case of infra-Bar (or any other mutant, since B by definition is the upper limit of variation of facet number).This upper limit has already been realized in modified infra-Bar but not in infra-Bar at 25°C. Therefore an increase in temperature would be expected to have less effect on facet number in the presence of the modifier than in its absence. However, other things being equal, it would be expected to decrease the coefficient of variation in the presence of the modifier.

In the case of double infra-Bar the presence of the modifier increases rather than decreases the coefficient of variation (table 5). It would appear therefore that an increase in temperature should cause a relatively greater increase in modified double infra-Bar than in modified infra-Bar, since in the former the upper limit of the potential range of facet number which the double-infra-Bar eye may have has not been realized. In other words, it seems likely on the above hypothesis that a $B^i B^i$ female having more than 344 facets (table 5) may be derived.

It is of interest to consider the data on Bar derived at the two different temperatures in the light of the above discussion. At 25°C the modifier causes a significant decrease in the coefficient of variation (table 2), although both stocks are inbred (but not isogenic with each other) and both were raised under identical conditions. It would appear therefore that the upper limit of facet number which the Bar genotype may express is approximately 315 in the males and 211 in the females (table 2). At 29°C the presence of the modifier increases the coefficient of variation relative to that of unmodified Bar at the same temperature. The decrease in the coefficient of variation in Bar at 29°C as compared with that at 25°C is due largely if not entirely to the much more accurate control of temperature at the higher level though possibly also because the lower limit of facet number in Bar has been reached. But note that despite the more accurate temperature control at 29°C the coefficient of variation of modified Bar has increased relative to that at 25°C. Consequently the change in relationship

between the values of the coefficients of variation of Bar and modified Bar at the two temperatures cannot be accredited to the great reduction in the variability of unmodified Bar at 29°C as compared with 25°C. The increased variability of modified Bar at 29°C may be explained (1) by the fact that its entire range of variation at this temperature (117–234, compare males in table 3) falls well within the potential range of Bar, which must extend at least from 33 to 315 for the males, and (2) by the interaction of the antagonistic effects of temperature and *m*(*B*).

As stated above, the modifier does not affect the mean facet number of either wild type or *ey*^{2}. It is perhaps worth noting that in wild type the upper limit of the range is increased in both sexes (table 5). The change is minute, but in view of the low range of variation of wild type even under extremes of temperature (MARGOLIS and ROBERTSON 1937 report average values for wild type of 770.4 at 18°C and 694.6 at 28°C for males and 847.6 at 18°C and 749.0 at 28°C for females), it is perhaps significant. In eyeless^2 not only is the upper limit of the range of variation greatly raised (table 5) but also the coefficient of variation. In view of these considerations, the statement that the modifier affects facet number only in the presence of the Bar "alleles" should perhaps be qualified to read "mean facet" number, for it seems very likely that it does affect the development of the eye in wild type and eyeless^2 although not in such a way as to modify the mean facet number.

SUMMARY

It was found that modifier of Bar (*m*(*B*)) is a semi-dominant mutant located in the second chromosome about seven crossover units to the left of plexus.

Evidence is offered which indicates that *m*(*B*) is a point mutation.

Facet counts of Bar, double-Bar, infra-Bar, double-infra-Bar, eyeless,^{2} and wild-type with and without the modifier were made. The data indicate that *m*(*B*) increases the mean facet number of each of the Bar "alleles" but does not affect that of eyeless^2 or wild type.

The data are discussed in the light of a hypothesis on the mode of development of the eye. The hypothesis appears to be consistent with the data.

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TRANSLOCATIONS IN MAIZE INVOLVING THE SHORT ARM OF CHROMOSOME I

E. G. ANDERSON
California Institute of Technology, Pasadena, California

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THE linkage map of chromosome 1, the longest of the chromosomes of maize, presents certain difficulties due to its extreme length and to the long distances between good marker genes. The leftmost known gene is *sr* (striate leaf). About 25 units to the right of *sr* there is a group of genes clustered within a few units of *ts2* (tassel seed) and *P* (pericarp color). Then follows a long region of more than 50 units to the next good marker gene, *br* (brachytic culm), broken only by the very unsatisfactory marker gene *as* (asynaptic). Beyond *br* are several not very satisfactory genes covering a long region of 75 or more units to *bm2* (brown midrib) which is the right-most known gene. The two long regions, *P-br* and *br-bm2*, will probably prove to be considerably longer than listed when new genes are found which will permit a more adequate measurement in shorter sections.

EMERSON (1939) has made a detailed study of the linkage relations within the group of genes clustered about *P* and has also determined their orientation with reference to the map as a whole. Omitting genes which will not be further discussed in this paper, the sequence is *sr-ts2-P-br*. The distances are about 25 units between *sr* and *ts2*, 1.3 between *ts2* and *P*, and upward of 50 units between *P* and *br*.

Studies involving translocations have shown the gene *sr* and the group near *P* to be well out on the short arm; those from *br* to *bm2* in the long arm. The translocations themselves can be readily divided into two groups, first those in the neighborhood of *sr* and *P* or in the *P-br* interval but remote from *br*, and second those in the neighborhood of *br* or in the *br-bm2* interval. So far as checked cytologically, these correspond with morphological locations on the short arm and long arm, respectively. This report will summarize the data on linkage and cytological relationships of the first group, those located in the short arm of the chromosome.

TRANSLOCATIONS INCLUDED

Of translocations on which linkage data are available, eight have been located on the leftward portion of the linkage map of chromosome 1. The history of six of these has been published previously (ANDERSON 1935). These are as follows: T 1–2b; T 1–3a, additional data given by EMERSON 1939 and ANDERSON and BRINK 1940; T 1–5b, additional data by EMERSON 1939; T 1–5c, additional data by EMERSON 1939; T 1–9a, additional data by ANDERSON 1938; T 1–9c, additional data by ANDERSON 1938, and by

EMERSON 1939. The remaining two have both been isolated from lines derived from X-ray treatment in which two translocations instead of one were found. In both cases there has been confusion due to the simultaneous presence of two translocations.

T 1–2c was isolated out of the stock of T 1–10b (ANDERSON 1935). The earlier linkage records show inconsistencies, some indicating a position to the left of *P*, some to the right. Later work was confined to a culture which was closely linked to *sr*. Tests showed that this one involved chromosome 2. Most of the earlier seed had been discarded, and tests of the few ears which remained showed only T 1–2c. So the stock of T 1–10b has probably been lost.

T 1–6c was isolated out of the stock listed as T 1–3c (ANDERSON 1935). After the discovery of a linkage of yellow endosperm with pericarp color in this stock, the linkage work was confined to the T 1–6c line. A check was made on some of the original diakinesis determinations. Four were found to be independent of the nucleolus, while one was attached. The determination of the chromosomes involved in the translocation originally listed as T 1–3c is therefore not complete and may not be correct. The translocation, however, has been recovered and will be further studied.

Of the above translocations, T 1–2c and T 1–9c have been utilized by BURNHAM and CARTLEDGE (1939) in studies on smut resistance.

CYTOLOGY

Cytological examination of the mid-prophase of meiosis was made on five of these translocations. The positions of the interchange of strands are recorded as decimal fractions of the distance from the centromere. Thus 1 S .3 indicates chromosome 1, short arm, three-tenths of the distance from the centromere to the end of the chromosome.

T 1–2b	1 S .4	2 S .6
T 1–2c	1 S .7	2 L .3
T 1–3a	1 S .25	3 L .2
T 1–6c	1 S .3	6 L .2+
T 1–9c	1 S .6	9 L .2+

DATA ON TRANSLOCATIONS

T 1–2c

The most distal translocation, T 1–2c, is at or near the extreme left end of the known map of chromosome 1. Backcross data involving *sr* are presented in table 1. Among a total of 1292 plants there were 18 crossovers between T and *sr*, or 1.4 percent. The three point backcross data which include *sr* and *P* give the following distribution:

o	Region 1	Region 2	Regions 1 and 2
363	5	105	2

suggesting the order T-*sr*-*P*.

TABLE 1

Backcross data involving T 1-2c and sr.

	PARENTAL COMBINATION		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL
$\frac{T++}{+\,sr\,P}$	59	54	1	0	22	17	1	0	154
$\frac{T\,sr+}{++P}$	152	98	2	2	40	26	1	0	321
$\frac{T+}{+\,sr}$	169	160	4	2					335
$\frac{T\,sr}{++}$	74	108	2	1					185
From EMERSON 1939									
$\frac{T+}{+\,sr}$	151	144	1	1					297

Table 2 presents the backcross data involving the genes *ts2* and *P*. The order indicated is T-*ts2*-*P*, in agreement with the much larger quantity of data (1436 plants) assembled by EMERSON (1939). The combined data on a total of 1569 plants show 16.8 percent of crossing over between T and *ts2* and about 1.0 percent between *ts2* and *P*.

TABLE 2

Backcross data involving T 1-2c and ts2.

	NORMAL PLANTS CROSSOVER REGIONS			TOTAL	TASSEL SEED PLANTS PARENTAL COMBINATION	CROSSOVERS *ts2*−*P*
	o	1	2			
$\frac{T++}{+\,ts2\,P}$	74	9	1	84	73	1
$\frac{T+P}{+\,ts2+}$	42	5	2	49	61	0

The cytological placement is about S .7. The linkage data give it a map position 1.4 units from *sr*, probably to the left. Thus it is probably the leftmost known point on the chromosome.

The other seven translocations included in this report all appear to be

to the right of *P*. Since the linear order is the same for each translocation, much of the three point backcross information assembled may be condensed into a few tables. Thus, tables 3, 4, 5, and 6 summarize the data on backcrosses of each of the translocations with *P br*, *br bm2*, *sr P*, and *ts2 P*, respectively.

Most of the data of table 6 and likewise of table 2 were collected from cultures homozygous for *sm* (salmon silk color) making it possible to

TABLE 3

Backcross progenies from $\frac{P + br}{+ T +}$.

TRANSLOCATION	NON CROSSOVERS		CROSSOVERS REGION 1		CROSSOVERS REGION 2		CROSSOVERS REGIONS 1, 2		TOTAL
T 1-2b	52	64	3	0	47	54	1	0	221
T 1-3a	47	57	12	8	38	18	8	7	195
T 1-5b	129	113	37	34	61	47	13	15	449
T 1-5c	113	105	43	26	36	35	11	15	384
T 1-9a	28	22	10	4	8	13	3	7	94
T 1-9c	24	26	0	2	17	20	1	1	91

TABLE 4

Backcross progenies from $\frac{T + +}{+ br\ bm2}$.

TRANSLOCATION	NON CROSSOVERS		CROSSOVERS REGION 1		CROSSOVERS REGION 2		CROSSOVERS REGIONS 1, 2		TOTAL
T 1-3a	31	17	11	19	18	19	10	11	136
T 1-5b	20	17	6	7	18	15	11	13	107
T 1-9a	8	7	7	7	11	6	4	4	54
T 1-9c	15	18	12	10	10	12	5	10	92

TABLE 5

Backcross progenies from $\frac{+ + T}{sr\ P\ +}$.

TRANSLOCATIONS	NON CROSSOVERS		CROSSOVERS REGION 1		CROSSOVERS REGION 2		CROSSOVERS REGIONS 1, 2		TOTAL
T 1-3a	22	21	15	6	6	5	0	1	76
T 1-5b	79	99	46	42	46	43	11	9	375
T 1-6c	141	107	46	34	14	10	3	0	355
T 1-9a	40	36	25	14	8	13	4	2	142
T 1-9c	45	18	8	11	0	1	0	0	83

TABLE 6

Backcross progenies from $\frac{+\ +\ T}{ts2\ P\ +}$.

	NORMAL PLANTS				TASSEL SEED PLANTS		
	++T	*+P+*	*+++*	TOTAL	*ts2 P*	*ts2+*	TOTAL
T 1-2b	483	4	34	521	617	6	623
T 1-3a	170	0	45	215	206	1	207
T 1-5b	166	2	45	213	273	3	276
T 1-6c	350	5	49	404	419	3	422
T 1-9a	316	5	79	400	382	5	387
T 1-9c	1228	3	6	1237	1365	4	1369

classify *P* by silk color as well as by pericarp color. This made it possible to check the pollen classification of all non-tassel seed crossovers between *ts2* and *P*.

T 1–2b

In addition to the data presented in tables 3 and 6, a backcross involving only *P* and the translocation gave five crossovers among a total of 376 plants. These data may be summarized as follows:

Table 3	221 plants	*P*-1.8-T-46.2-*br*
Table 6	521 plants	*ts2*-0.8-*P*-6.5-T
Additional	376 plants	*P*-1.3-T

The relative order is clearly indicated by the data from table 6 as *ts2*—*P*—T—*br*. The average of all 1118 plants places the translocation 3.8 units to the right of *P*. The observed cytological position is about S .4.

T 1–3a

Linkage data from tables 3, 4, 5 and 6 plus data published by EMERSON (1939) may be summarized as follows:

Table 3	195 plants	*P*-17.9-T-36.4-*br*
Table 4	136 plants	T-37.5-*br*
Table 5	76 plants	*sr*-29-*P*-16-T
Table 6	215 plants	*P*-20.9-T
EMERSON 1939	101 plants	*P*-11.9-T
EMERSON 1939	315 plants	*ts2*-1.3-*P*-20.6-T

The location of the translocation is between *P* and *br*. The distance from *P* is 18.7 units based upon a total of 902 plants: from *br* 36.9 units based upon a total of 331 plants. The cytological position is about S .25 or one quarter of the way out from the centromere on the short arm.

T 1–5b

Data from the preceding tables are as follows:

Table 3	449 plants	*P*-22.0-T-30.1-*br*
Table 4	107 plants	T-34.6-*br*
Table 5	375 plants	*sr*-28.8-*P*-29.1-T
Table 6	213 plants	*ts2*-0.9-*P*-21.1-T
EMERSON 1939	211 plants	*ts2*-1.4-*P*-18.5-T
EMERSON 1939	87 plants	*P*-35.6-T

The translocation is about midway between *P* and *br* on the linkage map. Based on a total of 1335 plants for the *P*-T region and 556 for the T-*br* region, the map becomes *P*—24.2—T—30.9—*br*. No cytological observations have been made.

T 1–5c

In addition to the data of table 3, other backcrosses totalling 230 plants gave 50 crossovers between *P* and the translocation. A small culture from EMERSON (1939) is added.

Table 3	384 plants	*P*-24.7-T-25.3-*br*
Additional	230 plants	*P*-21.9-T
EMERSON 1939	82 plants	*P*-18.3-T

This translocation is also about midway between *P* and *br*, the combined data giving the map position *P*—23.0—T—25.3—*br*.

The similarity of T 1–5b and T 1–5c is so great as to cause some suspicion of their identity. In chromosome 5 both are very closely linked with *bm*. An intercross of the two gave an F_1 with pollen substantially normal in appearance. In origin they go back to sister seeds subjected to X-ray treatment. Errors in records and in technique cannot be entirely excluded but are rendered improbable by the fact that the two lines were widely separated until the chromosome determinations were made. A closer test of their possible identity will be made before attempting to evaluate the evidence.

T 1–6c

In addition to the data of tables 5 and 6, a backcross culture of 127 plants gave 12 crossovers with *P*.

Table 5	355 plants	*sr*-23.4-*P*-7.6-T
Table 6	404 plants	*ts2*-1.2-*P*-12.1-T
Additional	127 plants	*P*-9.5-T

The map position of T 1–6c is to the right of *P* as shown by the data of tables 5 and 6. The total of 886 plants listed above included 88 crossovers between *P* and the translocation, or 9.9 percent.

T 1–9a

In addition to the information presented in the preceding tables, a backcross culture of 93 plants showed 25 crossovers with *P*.

Table 3	95 plants	*P*-25-T-32-*br*
Table 4	54 plants	T-41-*br*
Table 5	142 plants	*sr*-31.7-*P*-19.0-T
Table 6	400 plants	*ts2*-1.2-*P*-19.7-T
Additional	93 plants	*P*-27-T

The map position of the translocation is to the right of *P* as shown most clearly by the data of tables 5 and 6. This is also indicated by a backcross test for linkage of *sr* and *P* in a plant homozygous for the translocation. This backcross progeny of 114 plants showed 29 crossovers or about 25 percent. Obviously the translocation has not separated *sr* and *P*. On the basis of all data presented above, the map order and distances are *P*—21.2—T—35.6—*br*.

Two plants homozygous for the translocation but heterozygous for *wx* and *P* were backcrossed. The non-waxy seeds yielded 47 red ears and 18 white, the waxy seeds 19 red and 63 white. This is a clear case of linkage and indicates that *P* is now included in the same chromosome as *wx*. From previous data reported (ANDERSON 1938), the locus of the translocation must be in the long arm of chromosome 9. The gene *wx*, from the short arm near the centromere, must now be in the interchange chromosome bearing the centromere from chromosome 9—that is, interchange chromosome 9^1. The left end of chromsome 1, with the genes *sr*, *ts2*, and *P*, can be included in interchange chromosome 9^1 only if the locus of translocation in chromosome 1 is in the short arm. Thus, despite the lack of cytological observation, the translocation T 1–9a may be assigned to the short arm of chromosome 1 between the centromere and the locus of the gene *P*.

T 1–9c

Table 3	91 plants	*P*-4-T-43-*br*
Table 4	92 plants	T-40-*br*
Table 5	83 plants	*sr*-23-*P*-1-T
Table 6	1237 plants	*ts2*-0.24-*P*-0.5-T

The position of the translocation is shown to be to the right of *P* by the data of table 6. The distance from *P* may be computed as 0.8, since there were but 11 crossovers among a total of 1411 plants. In computing the crossing over between *ts2* and *P*, the tassel seed plants from table 6 may also be included, giving a total of 2606 plants of which only 7, or 0.27 percent, were crossovers. This is much less than the standard value 1.3 (EMERSON 1939) and indicates a partial suppression of crossing over. Even greater suppression is to be expected between *P* and the translocation, so the computed crossover value, 0.8, may really correspond to a much greater distance on the normal linkage map.

A number of plants heterozygous for *P* and *wx* but homozygous for the translocation were backcrossed to the double recessive. These gave the

following distribution: non-waxy seeds, 235 red+9 white ears; waxy seeds, 14 red+200 white ears; showing linkage with only five percent of crossing over. Cytological observation has placed the translocation in the longarm of 9 (9 L .2+) and in the short arm of 1 (1 S .6). The gene *wx* is in the reconstituted chromosome 9' which includes the distal portion of the short arm of chromosome 1 (ANDERSON 1938). The linkage of *wx* and *P* in plants homozygous for the translocation places *P* in the distal section to the left of the locus of translocation in chromosome 1. This confirms the conclusions drawn from the data of tables 5 and 6.

SUMMARY OF LINKAGE RELATIONS OF THE TRANSLOCATIONS

Table 7 presents in summary form the relation between the cytological

TABLE 7

Comparison of position of interchange with map location.

	CYTOLOGICAL POSITION	CROSSING OVER		
		T−*sr*	*P*−T	T−*br*
T 1-2c	S .7	1.4		
T 1-9c	S .6		0.8	
T 1-2b	S .4		3.8	
T 1-6c	S .3		9.9	
T 1-3a	S .25		18.7	36.9
T 1-9a	S		21.2	35.6
T 1-5b			24.2	30.9
T 1-5c			23.0	25.3

positions of interchange and the genetically determined map locations for the translocations involving the short arm of chromosome 1.

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CYTOGENETIC STUDIES OF A CASE OF POLLEN ABORTION IN MAIZE[1] *

C. R. BURNHAM
Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota

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IN inbred material grown for use as a standard normal stock, plants with partially sterile pollen were found. The inbreds used, obtained at the CALIFORNIA INSTITUTE of TECHNOLOGY indirectly from DR. DOLK in 1930, came from the CONNECTICUT AGRICULTURAL EXPERIMENT STATION. According to information furnished the writer by W. R. SINGLETON, three inbred lines were sent to DR. DOLK at that time: C677, a Cornell inbred from Bloody Butcher; 112-1D, a Leaming inbred with a very long slender ear; and C21, a Burr White inbred. No records are available to determine which one was used. It is recalled that the line used was a poor pollen shedder and had a fairly long ear. According to the writer's notes, the first outcross which was with a white strain had yellow endosperm. Therefore the line in which the partial sterility reported in this paper occurred is probably either C677 or 112-1D.

The number of plants examined in the original culture was small but all were partially sterile. One of these was used in reciprocal crosses with a normal stock. From the cross using it as the pollen parent, no partially sterile plants were found, while from the one using it as the female parent there was a ratio approximating one plant with partially sterile pollen: one plant with normal pollen. The partially sterile plants showed sterility only in the pollen, the ears being normally filled. Cytological examination showed no ring formation or other visible abnormality in the partially sterile plants.

A preliminary report on this case has been published (BURNHAM 1935) but a more detailed cytogenetic study of the material was made, since this is a type of sterility which would not appear to be at a disadvantage in selection under natural conditions or even under a planned breeding program. Poor pollen shedding is not characteristic of partially sterile plants in the outcrosses.

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* The first crosses were made at the CALIFORNIA INSTITUTE OF TECHNOLOGY and studies were continued at the UNIVERSITY OF MISSOURI, CORNELL UNIVERSITY, and WEST VIRGINIA UNIVERSITY. I wish to express my thanks to these institutions and particularly to DRS. E. G. ANDERSON, L. J. STADLER, and R. A. EMERSON for furnishing me with the facilities for continuing the studies. The detailed cytological study was completed at the MINNESOTA AGRICULTURAL EXPERIMENT STATION.

INHERITANCE OF POLLEN STERILITY IN CROSSES

A count of the pollen sterility on 24 plants shows wide variability (from 33 to 67.9 percent) with an average of 49.2 percent. Usually the defective pollen grains contain no starch or there is only a small percent partially filled, but occasionally the number of this type is much higher. This pollen abortion is ascribed to a gene *pa;* plants heterozygous for *pa* showing semisterile pollen but normally filled ears. The breeding behavior of plants heterozygous for *pa* (designated *pa*/+) was studied in progenies from self-pollination and from reciprocal crosses with normals (table 1). The ears on *pa*/+ plants from self-pollination and from crosses with normal

TABLE 1

Results from various matings of plants with semisterile pollen, these having originated from the cross of semisterile×normal.

TYPE OF CROSS	POLLEN SEMISTERILE	POLLEN NORMAL	TOTAL	DEV./S.E ON 1:1 BASIS
Normal×semisterile	0	219	219	14.8
Normal×semisterile (1940 data)	15*	225	240	13.6
Semisterile×normal	583†	547	1010	1.4
Semisterile selfed	180	167	347	0.7

* One additional plant was low sterile in pollen.

† Two additional plants were classified as low sterile. One of these was self-pollinated, the progeny containing 12 with typical semisterile pollen and 12 normal.

are completely filled, indicating that *pa* is not lethal to the female gametophyte. In the progeny of crosses of heterozygous *pa* female×normal male, the total numbers fit closely a ratio of one plant with semisterile pollen: one with normal pollen (table 1). Using the χ^2 test for homogeneity (MATHER 1938) on the 13 different small families which make up part of the total from this type of cross (table 1) P was between .1 and .05, indicating they belonged essentially to the same population. For the five larger families which make up the remainder of the total, P was between .3 and .2, likewise indicating a good fit.

The progeny from selfing heterozygous *pa* plants also fit closely a 1:1 ratio, as shown in table 1. Plants with 100 percent pollen sterility might be expected if *pa* pollen functioned, but none was found.

The earlier crosses (one made at California and three at Columbia, Missouri) indicated that *pa* was lethal in the pollen, since in the progeny of the cross of normal by heterozygous *pa* male there were no plants with semisterile pollen. However from three of four similar crosses made at Minnesota and grown in 1940, about eight percent of such plants was found (table 1). Unfortunately the supposedly normal female parents of the three crosses were not stocks that had been checked for sterility. In only

one of these crosses was it possible to determine satisfactorily that the ears were normal, thus eliminating the possibility in this case that the semisterile plants resulted from off-pollination involving a chromosomal interchange. In one case the same *pa*/+ plant was tested in two different crosses. From one of these there were three semisterile plants in a total of 63 while from the other cross (with a known normal stock) there was none in 49 plants. Using MULLER'S (1923) method of calculation, the chances of getting at least one in the population of 49, if 3 in 63 are taken as the expected, are 24:1. These odds are not high, but when taken with the results from earlier tests indicate that a further check of the semisterile offspring of such crosses is needed to establish the fact that *pa* male gametes may function. If it does occur, then it should be possible to obtain from self-pollination plants homozygous for the *pa* gene. Such plants would show 100 percent pollen sterility and when crossed with normals all the F_1 plants would be partially sterile. This may be the origin of the progeny in which *pa* was first found.

LINKAGE DATA

In order to determine the linkage group involved, plants heterozygous for *pa* were used as the female parents in crosses with factors in the different linkage groups. The resulting partially sterile F_1 plants were thus heterozygous for *pa* and for the other genetic factors. These were used as the pollen parents either with their sibs or with the recessive parent. In such tests deficient ratios for genes linked with *pa* would result because of the lethal effect of *pa* in the pollen. A direct linkage test was made in certain cases by using the above F_1 plants as the female parents in backcrosses to the recessives, classifying the resulting plants for pollen sterility and the known genes.

The factors and the linkage groups tested are listed below:

Linkage group 1. *P** (pericarp) *br** (brachytic) *f* (fine stripe) *bm2** (brown midrib)
Linkage group 2. *lg* (liguleless) *v4** (virescent) *Ch** (chocolate pericarp)
Linkage group 3. none tested
Linkage group 4. *Tu* (tunicate)
Linkage group 5. *bt* (brittle endosperm) *pr* (red aleurone)
Linkage group 6. *Y* (yellow endosperm)
Linkage group 7. *ra* (ramosa) *gl* (glossy)
Linkage group 8. *j* (japonica stripe)
Linkage group 9. *sh* (shrunken) *wx** (waxy endosperm)
Linkage group 10. *g* (golden)

Those factors marked with an asterisk were given both types of test for linkage. The *P br* factors in the first linkage group showed linkage with

sterility (table 2) and abnormal ratios for these same factors were observed when the partially sterile F_1 plants were used as the pollen parents (table 3). None of the other crosses gave any evidence of linkage or of abnormal ratios; although, with the exception of linkage group 2, the groups were not adequately tested over their entire length.

TABLE 2

Linkage data from plants heterozygous for pa and for factors in linkage group one crossed with p+br bm2, using the heterozygous plants with semisterile pollen as the female parents.

	REGION								
	0	1	2	3	1–2	1–3	2–3	1–2–3	TOTAL
GENOTYPE									
P + br bm2 / *+ pa + +*	25 32	15 6	15 13	21 30	7 9	13 16	10 7	8 5	232
+ + br bm2 / *P pa + +*	14 20	4 4	15 9	8 14	2 6	3 1	4 4	1 2	111
Total, including multiples		102	117	147	40	49	41		
Percentage recombination including multiples		29.7	34.1	42.9					

In table 2 are the four-point linkage data obtained with factors in the *P-br* linkage group. The sterility "factor," *pa*, is approximately midway between *P* and *br*, probably a little closer to *P*, the gene order and recombination values being: *P* 30 *pa* 34 *br* 43 *bm2*. There is no indication of reduction in crossing over in heterozygous *pa* plants such as was found for *sp2* (small pollen) in chromosome 10 (RHOADES and RHOADES 1939).

EFFECT ON GENETIC RATIOS

As mentioned above, one evidence that *pa* was located in linkage group 1 was the abnormal genetic ratios for certain genes in that group when plants heterozygous for *pa* and for these genes were used as pollen parents. The expected ratio depends on the strength of the linkage with *pa*. Data from matings involving heterozygous *pa* plants and their normal sibs are given in table 3. The recombination percentages of *pa* with each factor as indicated by the percent of recessives, assuming no transmission of *pa* through the pollen, are given in the sixth column. For self-pollination, with this assumption, the expected results may be stated as follows: (1) Where *pa* enters with the recessive allele, the expected percentage of recessives is one-half the recombination percentage; (2) where *pa* enters with the dominant the expected percentage of recessives is one-half the non-recombina-

TABLE 3

Segregation for factors in linkage group 1 of maize in different types of matings involving plants with semisterile pollen and their normal (non-sterile) sibs. The genotype of the semisterile plants was pa+/+y in which y was either P, br, f, or bm2; that of the normal sibs did not contain pa.

DESCRIPTION OF CROSS	CULTURE NO.	y = P						y = br						y = f						y = bm2				
		P	*p*	% *p*	EXPECTED % *p*	DEV. / S. E.	% RECOMB. WITH *pa*	+	*br*	% *br*	EXPECTED % *br*	DEV. / S. E.	% RECOMB. WITH *pa*	+	*f*	% *f*	EXPECTED % *f*	DEV. / S. E.	% RECOMB. WITH *pa*	+	*bm2*	% *bm2*	EXPECTED % *bm2*	DEV. / S. E.
Semisterile selfed	3192–8							116	66	36.3	25	3.5	27.4	126	58	31.5	25	2.0	37.0	142	42	22.8	25	0.7
Same	3191–12	176	10	5.4	25	6.1	10.8	125	50*	28.6	25	1.1	42.8	166	23	12.2	25	4.1	?	162	37	14.3	25	3.5
Normal *p* × semisterile sib	NX3191–12	54	14	20.6	50	4.9	20.6	44	24	35.3	25	2.0	34.4											
Normal recessive × semisterile	X3191–12	119	27	18.5	50	7.7	18.5																	
Same	X3864–3							108	289	72.8	50	9.1	27.2	138	259	65.2	50	6.1	34.8	211	186	46.9	50	1.2
Same	total of all this type	181	46	20.3	50	9.0	20.3	146	374	71.9	50	10.0	28.1	304	535	63.8	50	8.1	36.2	211	186	46.9	50	1.2
Semisterile × normal recessive	3864–3X							164	113	40.8	50	3.1	—	168	108	39.1	50	3.6	—	148	128	46.4	50	1.2
Same	total of all this type	120	121	50.2	50	0.6		335	276	45.2	50	2.4	—	680	543	44.4	50	4.0	—	322	292	47.6	50	1.2
Normal recessive × normal sib of semisterile								95	75	44.1	50	1.6	—	198	169	46.0	50	1.5	—	43	50	53.8	50	0.7
Normal sib of semisterile × normal recessive	3864–1X–N													151	158	51.1	50	0.4	—					

* Fourteen additional plants could not be classified with certainty for *br*.

tion percentage. With few exceptions, when the heterozygous *pa* plants are used as the pollen parent, the deviations from normal ratios are in the directions expected and are highly significant. One of these apparent exceptions is plant number 3191-12 which, when self-pollinated, shows significant deficiencies for *p* and for *f* and *bm2*, and a non-significant excess for *br* (table 3). Since *pa* came in with *p* and with *Br F Bm2*, the deficiency for *p* is expected, but *br* and *f* are expected to be in excess while the ratio for *bm2* should not be greatly affected because of its distance from *pa*. Since the additional crosses using 3191-12 (table 3) did not segregate either *f* or *bm2*, no additional tests of segregation for these factors are available. One of the additional crosses used 3191–12 as the male parent with a non-sterile sib (table 3). Here the excess of *br* corresponds to 34 percent recombination between *br* and *pa* and the deficiency of *p* corresponds to 21 percent recombination between *p* and *pa*. Another cross using 3191-12 as the male was a backcross for *P-p* only, and had a deficiency of *p* corresponding to about 19 percent recombination between *p* and *pa*. Since these tests show the usual effect of *pa* on the ratios, some other factor must have upset the results obtained from self-pollination of 3191-12. A second semisterile plant, when self-pollinated, showed the expected effect of *pa* (table 3).

One heterozygous *pa* plant, number 3864-3, was backcrossed reciprocally with normal *br f bm2* individuals (table 3). In the progeny obtained when used as the male, *br* and *f* were significantly in excess, as expected since *pa* entered with the normal alleles. In the reciprocal cross the normal classes were in excess. This excess of the normal alleles cannot be explained, but at least there is no indication of lethal action of *pa* in the ovules, since in that case the normal classes would have been deficient. The ratios for *bm2* were not affected by *pa* in either of these crosses.

In comparison with these results, the normal sibs of the heterozygous *pa* plants were tested as male and as female parents in backcrosses with *br f bm2* individuals. There were no significant deviations from normal ratios for these factors in any of these crosses (table 3).

The percentage of recombination between *pa* and the gene *P* indicated in these tests is lower in all cases than the 30 percent indicated by the direct linkage test. This is also true for three out of five such tests with *br*, the fourth one being the same, while only one has a higher value. The percentages of recombination are calculated by assuming no functioning of *pa* male gametes. However, since any functioning of *pa* male gametes would increase the apparent amount of recombination, the functioning of *pa* pollen cannot be the explanation. Variability in the ease of classifying *f* and *br* could be a possible explanation for the ratios involving these characters if the errors were mainly misclassifications of normals as recessives. In one cross of $+f \times pa+/+f$ a 1:1 segregation for $+:f$ was observed in the green-

house, where an excess of *f* was expected (in the seedling stage *f* was classified by its virescent characteristic). When the same cross was grown in the field, the *f* character showed so few stripes that satisfactory classification was impossible. Errors in this case would probably be in classifying *f* as +, in which case the recombination values again would be higher than those obtained by the direct test. Another possible explanation might be that a gametophyte factor affecting pollen tube growth was present.

Similar tests were set up in which *pa* entered with the *br f* chromosome. Only the segregation for *f* has been tested. One plant heterozygous for *pa*, its genotype being *pa f*/++, was used as the pollen parent on sibs not carrying *pa*. In a population of 237 plants 18.1 percent were *f*, a deficiency corresponding to 36.2 percent recombination between *pa* and *f*. This same plant, when self-pollinated gave 25.2 percent *f* in a population of 135 corresponding to 50 percent recombination. Application of the χ^2 test for goodness of fit to data from the latter cross to 18.1 percent *f* gives a P value of between .05 and .01 indicating that the observed segregation may not be a chance deviation from the expected deficiency of *f*. This result is similar to that obtained for 3191-12, details of which are given above. That this result from self-pollination does not always occur is shown by the selfed progeny of a *pa*/+ sib in which there were 17.2 percent of *f* plants in a population of 157 plants.

CYTOLOGICAL STUDY

Since the breeding behavior suggests a deficiency viable in the female gametophyte but not in the male, material was examined cytologically in detail at pachytene of microsporogenesis. In order to mark chromosome 1 cytologically for identification with certainty, heterozygous *pa* plants were crossed with the homozygous chromosomal interchange T1-6b reported by BURNHAM in 1932. Prophase studies of the plants were made, followed by later pollen classification. The plants carrying both *pa* and T1-6b had about 70 percent abortion while those with only T1-6b had about 25 percent. Detailed study of chromosome 1 in the two groups of plants showed no visible abnormality in either group, indicating no sizable deficiency associated with *pa*. A very minute deficiency might be present without being detected. Since there is no visible chromosome abnormality *pa* may be considered as a gene, realizing that this is not a certain criterion of separation.

A genetic test for deficiency has not been set up. The only gene in the neighborhood of *pa* is *as* (asynaptic) which is male sterile in homozygous condition (BEADLE 1930).

DISCUSSION

STADLER (1933) has described an X-ray induced deficiency in chromo-

some 10 of maize which was transmitted through only a portion of the ovules and not at all through the pollen. SINGLETON and MANGELSDORF (1940) described a naturally occurring genetic condition in chromosome 4, producing small but well-filled pollen (*sp*), which infrequently functioned in competition with normal pollen, and a variable lethality of ovules.

A similar case has been reported in chromosome 10 of maize, small pollen *sp2* (RHOADES and RHOADES 1939). In neither *sp* nor *sp2* could a deficiency be seen in the meiotic prophase chromosomes.

The case reported in this paper differs in that there is no inviability of *pa* ovules. Also *pa* pollen is probably non-functional or nearly so, the grains being for the most part empty or only partially filled with starch. The case is similar to *sp* in that no abnormality could be found cytologically. Since *pa* is not lethal to ovules, and the ears as a result are normally filled, genes of this type would not be weeded out by natural selection. This is indicated by the apparent fact that *pa* was carried along in one of the established inbred lines. To what extent different genes of this type can be accumulated in a plant is not known.

In a study of smut resistance using chromosomal interchanges (BURNHAM and CARTLEDGE 1939), the minimum number of pairs of genetic factors for smut reaction required to explain the results is six, and the maximum number is much larger. Except in one or two cases a definite position for these smut factors could not be assigned. Every interchange marks a point of breakage in each of two chromosomes. Each of these two points of breakage must be checked in the same inheritance study by using a different interchange with one locus near the one being checked. In studying characters which are complex in inheritance, the breakage point in the new interchange being used as a check may be near the locus of another genetic factor affecting the character being studied. Since genes of the *pa* type appear to produce no extreme morphological changes in plants carrying them and only one linkage group is involved, they should prove useful in supplementing the chromosomal interchanges and inversions for studies of the inheritance of smut resistance, earliness, yield, and similar characters.

SUMMARY AND CONCLUSIONS

A gene for pollen abortion *pa* was found in an established inbred line. Plants heterozygous for this gene have semisterile pollen, but normal ears. This gene is lethal, or nearly so, to pollen carrying it; but has no lethal effect on the ovules. As a result *pa* is transmitted mainly, if not entirely, through the ovules.

The gene is located in the first linkage group, between *P* and *br*, the order and recombination values from direct linkage tests being: *P* 30 *pa* 34 *br*.

Abnormal ratios for genes linked with *pa* were observed when plants heterozygous for *pa* and for these genes were self-pollinated or used as the pollen parents in sib crosses or backcrosses.

No cytological abnormality could be located in chromosome 1 of plants carrying *pa*.

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New Publications Received

COLIN, EDWARD C. *Elements of Genetics.* Pp. xii+386. Figs. 47. The Blakiston Co. Philadelphia, 1941. $3.

ELLINGER, FRIEDRICH. *The biologic fundamentals of radiation therapy.* English translation by Reuben Gross. Pp. xvi+360. Figs. 79. Nordeman Publishing Co. New York. $5.00.

FELT, EPHRAIM PORTER. Pruning trees and shrubs. Pp. 237. Fig. 94. Orange Judd Pub. Co. New York, 1941. $2.00.

POOL, RAYMOND J. *Flowers and flowering plants.* Second Edition. Pp. xxiii+428. Fig. 210. McGraw-Hill Book Co. New York, 1941. $3.50.

SHULL, A. FRANKLIN, GEORGE R. LA RUE, ALEXANDER G. RUTHVEN. *Principles of animal biology.* 5th Ed. Pp. xiv+417. Fig. 298. McGraw-Hill Company, Inc. New York. 1941. $3.50.

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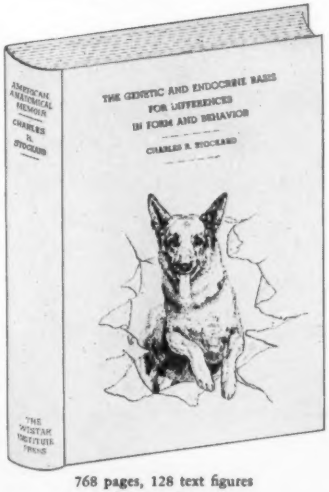

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